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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/82, 15/54, C08B 30/04, A01H 5/00

A2

(11) International Publication Number: WO 96/34968

(43) International Publication Date: 7 November 1996 (07.11.96)

(21) International Application Number: PCT/GB96/01075

(22) International Filing Date: 3 May 1996 (03.05.96)

(30) Priority Data:

9509229.2	5 May 1995 (05.05.95)	GB
9607409.1	10 April 1996 (10.04.96)	GB

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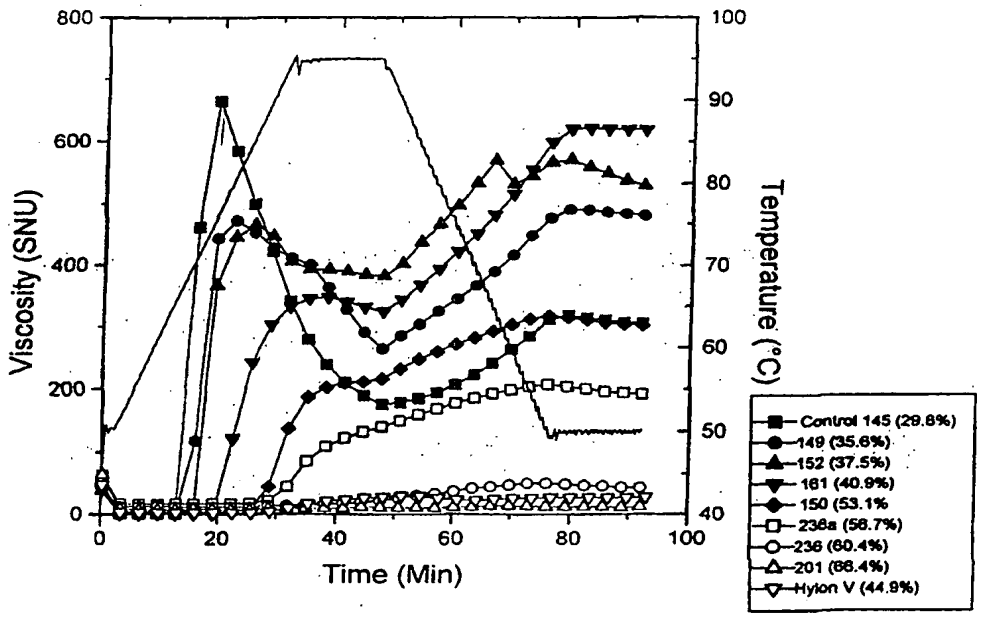
(81) Designated States: AU, BR, CA, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: IMPROVEMENTS IN OR RELATING TO PLANT STARCH COMPOSITION



(57) Abstract

Disclosed is a nucleotide sequence encoding an effective portion of a class A starch branching enzyme (SBE) obtainable from potato plants, or a functional equivalent thereof, together with, inter alia, a corresponding polypeptide, a method of altering the characteristics of a plant, a plant having altered characteristics; and starch, particularly starch obtained from a potato plant, having novel properties.

Title: Improvements in or Relating to Plant Starch Composition

Field of the Invention

This invention relates to novel nucleotide sequences, polypeptides encoded thereby, vectors and host cells and host organisms comprising one or more of the novel sequences, and to a method of altering one or more characteristics of an organism. The invention al;so relates to starch having novel properties and to uses thereof.

Background of the Invention

Starch is the major form of carbon reserve in plants, constituting 50% or more of the dry weight of many storage organs - e.g. tubers, seeds of cereals. Starch is used in numerous food and industrial applications. In many cases, however, it is necessary to modify the native starches, via chemical or physical means, in order to produce distinct properties to suit particular applications. It would be highly desirable to be able to produce starches with the required properties directly in the plant, thereby removing the need for additional modification. To achieve this via genetic engineering requires knowledge of the metabolic pathway of starch biosynthesis. This includes characterisation of genes and encoded gene products which catalyse the synthesis of starch. Knowledge about the regulation of starch biosynthesis raises the possibility of "re-programming" biosynthetic pathways to create starches with novel properties that could have new commercial applications.

The commercially useful properties of starch derive from the ability of the native granular form to swell and absorb water upon suitable treatment. Usually heat is required to cause granules to swell in a process known as gelatinisation, which has been defined (W A Atwell *et al*, Cereal Foods World *33*, 306-311, 1988) as "... *the collapse (disruption) of molecular orders within the starch granule manifested in irreversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence, and starch solubilisation. The point of initial gelatinisation and the range over which it occurs is governed by starch concentration, method of observation, granule type, and heterogeneities within the granule population under observation*". A number of techniques are available

cited above) as "*...a process which occurs when the molecules comprising gelatinised starch begin to reassociate in an ordered structure...*". At 50°C, it is primarily the amylose component which reassociates, as indicated by the increase in viscoamylograph viscosity for starch from normal maize (21.6% amylose) compared with starch from waxy maize (1.1% amylose) as shown in Figure 2. Figure 2 is a viscoamylograph of 10%w/w starch suspensions from waxy maize (solid line), conventional maize (dots and dashes), high amylose variety (hylon 5, dotted line) and a very high amylose variety (hylon 7, crosses). The temperature profile is also shown by a solid line, as in Figure 1. The extent of viscosity increase in the viscoamylograph on cooling and holding at 50°C depends on the amount of amylose which is able to reassociate due to its exudation from starch granules during the gelatinisation and pasting processes. A characteristic of amylose-rich starches from maize plants is that very little amylose is exuded from granules by gelatinisation and pasting up to 95°C, probably due to the restricted swelling of the granules. This is illustrated in Figure 2 which shows low viscosities for a high amylose (44.9%) starch (Hylon 5) from maize during gelatinisation and pasting at 95°C and little increase in viscosity on cooling and holding at 50°C. This effect is more extreme for a higher amylose content (58%, as in Hylon 7), which shows even lower viscosities in the viscoamylograph test (Figure 2). For commercially-available high amylose starches (currently available from maize plants, such as those described above), processing at greater than 100°C is usually necessary in order to generate the benefits of high amylose contents with respect to increased rates and strengths of reassociation, but use of such high temperatures is energetically unfavourable and costly. Accordingly, there is an unmet need for starches of high amylose content which can be processed below 100°C and still show enhanced levels of reassociation, as indicated for example by viscoamylograph measurements.

The properties of potato starch are useful in a variety of both food and non-food (paper, textiles, adhesives etc.) applications. However, for many applications, properties are not optimum and various chemical and physical modifications well known in the art are undertaken in order to improve useful properties. Two types of property manipulation which would be of use are: the controlled alteration of gelatinisation and pasting temperatures; and starches which suffer less granular fragmentation during pasting than

between the two classes. One general distinction of note would appear to be the presence, in class A SBE molecules, of a flexible N-terminal domain, which is not found in class B molecules. The distinctions noted by Burton *et al.* are relied on herein to define class A and class B SBE molecules, which terms are to be interpreted accordingly.

However in potato, only one isoform of the SBE molecule (belonging to class B) has thus far been reported and only one gene cloned (Blennow & Johansson, 1991 Phytochem. *30*, 437-444, and Koßmann *et al.*, 1991 Mol. Gen. Genet. *230*, 39-44). Further, published attempts to modify the properties of starch in potato plants (by preventing expression of the single known SBE) have generally not succeeded (e.g. Müller-Rober & Koßmann 1994 Plant Cell and Environment *17*, 601-613).

Summary of the Invention

In a first aspect the invention provides a nucleotide sequence encoding an effective portion of a class A starch branching enzyme (SBE) obtainable from potato plants.

Preferably the nucleotide sequence encodes a polypeptide comprising an effective portion of the amino acid sequence shown in Figure 5 (excluding the sequence MNKRIDL, which does not represent part of the SBE molecule), or a functional equivalent thereof (which term is discussed below). The amino acid sequence shown in Figure 5 (Seq ID No. 15) includes a leader sequence which directs the polypeptide, when synthesised in potato cells, to the amyloplast. Those skilled in the art will recognise that the leader sequence is removed to produce a mature enzyme and that the leader sequence is therefore not essential for enzyme activity. Accordingly, an "effective portion" of the polypeptide is one which possesses sufficient SBE activity to complement the branching enzyme mutation in *E. coli* KV 832 cells (described below) and which is active when expressed in *E. coli* in the phosphorylation stimulation assay. An example of an incomplete polypeptide which nevertheless constitutes an "effective portion" is the mature enzyme lacking the leader sequence. By analogy with the pea class A SBE sequence, the potato class A sequence shown in Figure 5 probably possesses a leader sequence of about 48 amino acid residues, such that the N terminal amino acid sequence is thought to commence around the glutamic acid residue (E) at position 49 (EKSSYN... etc.). Those skilled in the art will appreciate

sequence. Accordingly, functionally equivalent sequences will also include those sequences which can hybridise, under stringent hybridisation conditions, to the sequence of the invention (rather than the complement thereof). Such "antisense" equivalents will preferably possess at least 85%, more preferably at least 90%, and most preferably 95% sequence homology with the complement of the sequence of the invention as exemplified by nucleotides 289 to 2790 of the DNA sequence shown in Figure 5. Particular functional equivalents are shown, for example, in Figures 8 and 10 (if one disregards the various frameshift mutations noted therein).

The invention also provides vectors, particularly expression vectors, comprising the nucleotide sequence of the invention. The vector will typically comprise a promoter and one or more regulatory signals of the type well known to those skilled in the art. The invention also includes provision of cells transformed (which term encompasses transduction and transfection) with a vector comprising the nucleotide sequence of the invention.

The invention further provides a class A SBE polypeptide, obtainable from potato plants. In particular the invention provides the polypeptide in substantially pure form, especially in a form free from other plant-derived (especially potato plant-derived) components, which can be readily accomplished by expression of the relevant nucleotide sequence in a suitable non-plant host (such as any one of the yeast strains routinely used for expression purposes, e.g. *Pichia spp*. or *Saccharomyces spp*). Typically the enzyme will substantially comprise the sequence of amino acid residues 49 to 882 shown in Figure 5 (disregarding the sequence MNKRIDL, which is not part of the enzyme), or a functional equivalent thereof. The polypeptide of the invention may be used in a method of modifying starch *in vitro*, comprising treating starch under suitable conditions (e.g. appropriate temperature, pH, etc) with an effective amount of the polypeptide according to the invention.

The term "functional equivalent", as applied herein to amino acid sequences, is intended to encompass amino acid sequences substantially similar to that shown in Figure 5, such that the polypeptide possesses sufficient activity to complement the branching enzyme mutation in *E. coli* KV 832 cells (described below) and which is active in *E. coli* in the

at least one third of the full length sequence, but by simple trial and error other fragments (smaller or larger) may be found which are functional in altering the characteristics of the plant.

Thus, in a further aspect the invention provides a method of altering the characteristics of a plant, comprising introducing into the plant an effective portion of the sequence of the invention operably linked to a suitable promoter active in the plant. Conveniently the sequence will be linked in the anti-sense orientation to the promoter. Preferably the plant is a potato plant. Conveniently, the characteristic altered relates to the starch content and/or starch composition of the plant (i.e. amount and/or type of starch present in the plant). Preferably the method of altering the characteristics of the plant will also comprise the introduction of one or more further sequences, in addition to an effective portion of the sequence of the invention. The introduced sequence of the invention and the one or more further sequences (which may be sense or antisense sequences) may be operably linked to a single promoter (which would ensure both sequences were transcribed at essentially the same time), or may be operably linked to separate promoters (which may be necessary for optimal expression). Where separate promoters are employed they may be identical to each other or different. Suitable promoters are well known to those skilled in the art and include both constitutive and inducible types. Examples include the CaMV 35S promoter (e.g. single or tandem repeat) and the patatin promoter. Advantageously the promoter will be tissue-specific. Desirably the promoter will cause expression of the operably linked sequence at substantial levels only in the tissue of the plant where starch synthesis and/or starch storage mainly occurs. Thus, for example, where the sequence is introduced into a potato plant, the operably linked promoter may be tuber-specific, such as the patatin promoter.

Desirably, for example, the method will also comprise the introduction of an effective portion of a sequence encoding a class B SBE, operably linked in the antisense orientation to a suitable promoter active in the plant. Desirably the further sequence will comprise an effective portion of the sequence encoding the potato class B SBE molecule. Conveniently the further sequence will comprise an effective portion of the sequence described by Blennow & Johansson (1991 Phytochem. *30*, 437-444) or that disclosed in

chips (amylose generally being used as a coating to prevent fat uptake), and to prepare mashed potato (especially "instant" mashed potato) having particular characteristics.

In particular relation to potato plants, the invention provides a potato plant or part thereof which, in its wild type possesses an effective SBE A gene, but which plant has been altered such that there is no effective expression of an SBE A polypeptide within the cells of at least part of the plant. The plant may have been altered by the method defined above, or may have been selected by conventional breeding to be deleted for the class A SBE gene, presence or absence of which can be readily determined by screening samples of the plants with a nucleic acid probe or antibody specific for the potato class A gene or gene product respectively.

The invention also provides starch extracted from a plant altered by the method defined above, or the progeny of such a plant, the starch having altered properties compared to starch extracted from equivalent, but unaltered, plants. The invention further provides a method of making altered starch, comprising altering a plant by the method defined above and extracting therefrom starch having altered properties compared to starch extracted from equivalent, but unaltered, plants. Use of nucleotide sequences in accordance with the invention has allowed the present inventors to produce potato starches having a wide variety of novel properties.

In particular the invention provides the following: a plant (especially a potato plant) altered by the method defined above, containing starch which, when extracted from the plant, has an elevated endotherm peak temperature as judged by DSC, compared to starch extracted from a similar, but unaltered, plant; a plant (especially a potato plant) altered by the method defined above, containing starch which, when extracted from the plant, has an elevated viscosity onset temperature (conveniently elevated by 10 - 25°C) as judged by viscoamylograph compared to starch extracted from a similar, but unaltered, plant; a plant (especially a potato plant) altered by the method defined above, containing starch which, when extracted from the plant, has a decreased peak viscosity (conveniently decreased by 240 - 700SNUs) as judged by viscoamylograph compared to starch extracted from a similar, but unaltered, plant; a plant (especially a potato plant) altered by the method

phase (step 5) and has a set-back viscosity of 303 SNUs or less as judged by viscoamylograph according to the protocol defined below.

For the purposes of the present invention, viscoamylograph conditions are understood to pertain to analysis of a 10% (w/w) aqueous suspension of starch at atmospheric pressure, using a Newport Scientific Rapid Visco Analyser with a heating profile of: holding at 50°C for 2 minutes (step 1), heating from 50 to 95°C at a rate of 1.5°C per minute (step 2), holding at 95°C for 15 minutes (step 3), cooling from 95 to 50°C at a rate of 1.5°C per minute (step 4), and then holding at 50°C for 15 minutes (step 5). Peak viscosity may be defined for present purposes as the maximum viscosity attained during the heating phase (step 2) or the holding phase (step 3) of the viscoamylograph. Pasting viscosity may be defined as the viscosity attained by the starch suspensions at the end of the holding phase (step 3) of the viscoamylograph. Set-back viscosity may be defined as the viscosity of the starch suspension at the end of step 5 of the viscoamylograph.

In yet another aspect the invention provides starch from a potato plant having an apparent amylose content (% w/w) of at least 35%, as judged by iodometric assay according to the method described by Morrison & Laignelet (1983 J. Cereal Science *1*, 9-20). Preferably the starch will have an amylose content of at least 40%, more preferably at least 50%, and most preferably at least 66%. Starch obtained directly from a potato plant and having such properties has not hitherto been produced. Indeed, as a result of the present invention, it is now possible to generate *in vivo* potato starch which has some properties analogous to the very high amylose starches (e.g. Hylon 7) obtainable from maize.

Starches with high (at least 35%) amylose contents find commercial application as, amongst other reasons, the amylose component of starch reassociates more strongly and rapidly than the amylopectin component during retrogradation processes. This may result, for example, in pastes with higher viscosities, gels of greater cohesion, or films of greater strength for starches with high (at least 35%) compared with normal (less than 35%) amylose contents. Alternatively, starches may be obtained with very high amylose contents, such that the granule structure is substantially preserved during heating, resulting in starch suspensions which demonstrate substantially no increase in viscosity during

Under the heading of "Industrial" applications, the novel starches of the invention may be advantageously employed, for example, in corrugating adhesives, in biodegradable products such as loose fill packaging and foamed shapes, and in the production of glass fibers and textiles.

Those skilled in the art will appreciate that the novel starches of the invention may, if desired, be subjected *in vitro* to conventional enzymatic, physical and/or chemical modification, such as cross-linking, introduction of hydrophobic groups (e.g. octenyl succinic acid, dodecyl succinic acid), or derivatization (e.g. by means of esterification or etherification).

In yet another aspect the invention provides high (35% or more) amylose starches which generate paste viscosities greater than those obtained from high amylose starches from maize plants after processing at temperatures below 100°C. This provides the advantage of more economical starch gelatinisation and pasting treatments through the use of lower processing temperatures than are currently required for high amylose starches from maize plants.

The invention will now be further described by way of illustrative example and with reference to the drawings, of which:

Figure 1 shows a typical viscoamylograph for a 10% w/w suspension of potato starch;

Figure 2 shows vsicoamylographs for 10% suspensions of starch from various maize varieties;

Figure 3 is a schematic representation of the cloning strategy used by the present inventors;

Figure 4a shows the amino acid alignment of the C-terminal portion of starch branching enzyme isoforms from various sources; amino acid residues matching the consensus

Examples

Example 1

Cloning of Potato class A SBE

The strategy for cloning the second form of starch branching enzyme from potato is shown in Figure 3. The small arrowheads represent primers used by the inventors in PCR and RACE protocols. The approximate size of the fragments isolated is indicated by the numerals on the right of the Figure. By way of explanation, a comparison of the amino acid sequences of several cloned plant starch branching enzymes (SBE) from maize (class A), pea (class A), maize (class B), rice (class B) and potato (class B), as well as human glycogen branching enzyme, allowed the inventors to identify a region in the carboxy-terminal one third of the protein which is almost completely conserved (GYLNFMGNEFGHPEWIDFPR) (Figure 4a). A multiple alignment of the DNA sequences (human, pea class A, potato class B, maize class B, maize class A and rice class B, respectively) corresponding to this region is shown in Figure 4b and was used to design an oligo which would potentially hybridize to all known plant starch branching enzymes: AATTT(C/T)ATGGGIAA(C/T)GA(A/G)TT(C/T)GG (Seq ID No. 20).

Library PCR

The initial isolation of a partial potato class A SBE cDNA clone was from an amplified potato tuber cDNA library in the λZap vector (Stratagene). One half μL of a potato cDNA library (titre 2.3 x 10^9pfu/mL) was used as template in a 50 μL reaction containing 100 pmol of a 16 fold degenerate POTSBE primer and 25 pmol of a T7 primer (present in the λZap vector 3' to the cDNA sequences - see Figure 3), 100 μM dNTPs, 2.5 U Taq polymerase and the buffer supplied with the Taq polymerase (Stratagene). All components except the enzyme were added to a 0.5 mL microcentrifuge tube, covered with mineral oil and incubated at 94°C for 7 minutes and then held at 55°C, while the Taq polymerase was added and mixed by pipetting. PCR was then performed by incubating for 1 min at 94°C, 1 min at 58°C and 3 minutes at 72°C. for 35 cycles. The PCR products were extracted with phenol/chloroform, ethanol precipitated and resuspended in TE pH 8.0 before cloning into the T/A cloning vector pT7BlueR (Invitrogen).

primer. Excess primers were removed on a Centricon 100 column and cDNA was recovered and precipitated with isopropanol. cDNA was A-tailed in a volume of 20 μl using 10 units terminal transferase (BRL), 200 μM dATP for 10 min at 37°C, followed by 5 min at 65°C. The reaction was then diluted to 0.5 ml with TE pH 8 and stored at 4°C as the cDNA pool. cDNA clones were isolated by PCR amplification using the primers $R_oR_idT_{17}$, R_o and POTSBE24. The PCR was performed in 50 μL using a hot start technique: 10 μL of the cDNA pool was heated to 94°C in water for 5 min with 25 pmol POTSBE24, 25 pmol R_o and 2.5 pmol of $R_oR_idT_{17}$ and cooled to 75°C. Five μL of 10 x PCR buffer (Stratagene), 200 μM dNTPs and 1.25 units of Taq polymerase were added, the mixture heated at 45°C for 2 min and 72°C for 40 min followed by 35 cycles of 94°C for 45 sec, 50°C for 25 sec, 72°C for 1.5 min and a final incubation at 72°C for 10 min. PCR products were separated by electrophoresis on 1% low melting agarose gels and the smear covering the range 600-800 bp fragments was excised and used in a second PCR amplification with 25 pmol of R_i and POTSBE25 primers in a 50 μL reaction (28 cycles of 94°C for 1 min, 50°C 1 min, 72°C 2 min). Products were purified by chloroform extraction and cloned into pT7 Blue. PCR was used to screen the colonies and the longest clones were sequenced.

The first round of RACE only extended the length of the SBE sequence approximately 100 bases, therefore a new A-tailed cDNA library was constructed using the class A SBE specific oligo POTSBE24 (10 pmol) in an attempt to recover longer RACE products. The first and second round PCR reactions were performed using new class A SBE primers (POTSBE 28 and 29 respectively) derived from the new sequence data. Conditions were as before except that the elongation step in the first PCR was for 3 min and the second PCR consisted of 28 cycles at 94 °C for 45 seconds, 55 °C for 25 sec and 72 °C for 1 min 45 sec.

Clones ranging in size from 400 bp to 1.4 kb were isolated and sequenced. The combined sequence of the longest RACE products and cDNA clones predicted a full length gene of about 3150 nucleotides, excluding the poly(A) tail (psbe 2con.seq in Fig. 8).

As the sequence of the 5' half of the gene was compiled from the sequence of several

degree of similarity, identical residues being denoted by the common letter, conservative changes by two dots and neutral changes by a single dot. Dashes indicate gaps introduced to optimise the alignment. The class A SBE protein has 44% identity over the entire length with potato class B SBE, and 56% identity therewith in the central conserved domain (Figure 6), as judged by the "Megalign" program (DNASTAR). However, Figure 7 shows a comparison between potato class A SBE (top row, residues 1-873) and pea class A SBE (bottom row, residues 1-861), from which it can be observed that cloned potato gene is more homologous to the class A pea enzyme, where the identity is 70 % over nearly the entire length, and this increases to 83 % over the central conserved region (starting at IPPP at position ~170). It is clear from this analysis that this cloned potato SBE gene belongs to the class A family of SBE genes.

An *E. coli* culture, containing the plasmid pSJ78 (which directs the expression of a full length potato SBE Class A gene), has been deposited (on 3rd January 1996) under the terms of the Budapest Treaty at The National Collections of Industrial and Marine Bacteria Limited (23 St Machar Drive, Aberdeen, AB2 1RY, United Kingdom), under accession number NCIMB 40781. Plasmid pSJ78 is equivalent to clone 19 described above. It represents a full length SBE A cDNA blunt-end ligated into the vector pBSSKIIP.

Polymorphism of class A SBE genes

Sequence analysis of the other two full length class A SBE genes showed that they contain frameshift mutations and are therefore unable to encode full length proteins and indeed they were unable to complement the branching enzyme deficiency in the KV832 mutant (described below). An alignment of the full length DNA sequences is shown in Figure 8: "10con.seq" (Seq ID No. 12), "19con.seq" (Seq ID No. 14) and "11con.seq" (Seq ID No. 13) represent the sequence of full length clones 10, 19 and 11 obtained by PCR using the PBER1 and PBERT primers (see below), whilst "psbe2con.seq" (Seq ID No. 18) represents the consensus sequence of the RACE clones and cDNA clone 3.2.1. Those nucleotides which differ from the overall consensus sequence (not shown) are shaded. Dashes indicate gaps introduced to optimise the alignment. Apart from the frameshift mutations these clones are highly homologous. It should be noted that the 5' sequence of psbe2con is longer because this is the longest RACE product and it also contains several

A comparison of all the full length sequences is shown in Figure 10. In addition to clones 10, 11 and 19 are shown the sequences of a *Bgl* II - *Xho* I product cloned directly into the QE32 expression vector ("86CON.SEQ", Seq ID No. 16) and the consensus sequence of the directly sequenced PCR products ("pcrsbe2con.seq", Seq ID No. 17). Those nucleotides which differ from the consensus sequence (not shown) are shaded. Dashes indicate gaps introduced to optimise the alignment. There are 11 nucleotide differences predicted to be present in the mRNA population, which are indicated by asterisks above and below the sequence. The other differences are probably PCR artefacts or possibly sequencing errors.

Complementation of a branching enzyme deficient *E. coli* mutant

To determine if the isolated SBE gene encodes an active protein i.e. one that has branching enzyme activity, a complementation test was performed in the *E. coli* strain KV832. This strain is unable to make bacterial glycogen as the gene for the glycogen branching enzyme has been deleted (Keil *et al.*, 1987 Mol. Gen. Genet. *207*, 294-301). When wild type cells are grown in the presence of glucose they synthesise glycogen (a highly branched glucose polymer) which stains a brown colour with iodine, whereas the KV832 cells make only a linear chain glucose polymer which stains blueish green with iodine. To determine if the cloned SBE gene could restore the ability of the KV832 cells to make a branched polymer, the clone pSJ90 (Seq ID No. 19) was used and constructed as below. The construct is a PCR-derived, substantially full length fragment (made using primers PBE 2B and PBE 2X, detailed below), which was cut with *Bgl* II and *Xho* I and cloned into the *Bam*H I / *Sal* I sites of the His-tag expression vector pQE32 (Qiagen). This clone, pSJ86, was sequenced and found to have a frameshift mutation of two bases in the 5' half of the gene. This frameshift was removed by digestion with *Nsi* I and *Sna*B I and replaced with the corresponding fragment from a Taq-generated PCR clone to produce the plasmid pSJ90 (sequence shown in Figure 12; the first 10 amino acids are derived from the expression vector). The polypeptide encoded by pSJ90 would be predicted to correspond to amino acids 46-882 of the full SBE coding sequence. The construct pSJ90 was transformed into the branching enzyme deficient KV832 cells and transformants were grown on solid PYG medium (0.85% KH_2PO_4, 1.1% K_2HPO_4, 0.6% yeast extract) containing 1.0% glucose. To test for complementation, a loop of cells was

phosphorylation stimulation assay and that the cloned gene does indeed code for a protein with branching enzyme activity.

Oligonucleotides

The following synthetic oligonucleotides (Seq ID No.s 1-11 respectively) were used:

$R_OR_IdT_{17}$	AAGGATCCGTCGACATCGATAATACGACTCACTATAGGGA$(T)_{17}$
R_O	AAGGATCCGTCGACATC
R_I	GACATCGATAATACGAC
POTSBE24	CATCCAACCACCATCTCGCA
POTSBE25	TTGAGAGAAGATACCTAAGT
POTSBE28	ATGTTCAGTCCATCTAAAGT
POTSBE29	AGAACAACAATTCCTAGCTC
PBER 1	GGGGCCTTGAACTCAGCAAT
PBERT	CGTCCCAGCATTCGACATAA
PBE 2B	CTTGGATCCTTGAACTCAGCAATTTG
PBE 2X	TAACTCGAGCAACGCGATCACAAGTTCGT

Example 2

Production of Transgenic Plants

Construction of plant transformation vectors with antisense starch branching enzyme genes

A 1200 bp *Sac* I - *Xho* I fragment, encoding approximately the -COOH half of the potato class A SBE (isolated from the rescued λZap clone 3.2.1), was cloned into the *Sac* I - *Sal* I sites of the plant transformation vector pSJ29 to create plasmid pSJ64, which is illustrated schematically in Figure 11. In the figure, the black line represents the DNA sequence. The broken line represents the bacterial plasmid backbone (containing the origin of replication and bacterial selection marker), which is not shown in full. The filled triangles on the line denote the T-DNA borders (RB = right border, LB = left border). Relevant restriction sites are shown above the black line, with the approximate distances (in kilobases) between the sites (marked by an asterisk) given by the numerals below the

Transformation was confirmed in all cases by production of a DNA fragment from the antisense gene after PCR in the presence of appropriate primers and a crude extract of genomic DNA from each regenerated shoot.

Characterisation of starch from potato plants

Starch was extracted from plants as follows: potato tubers were homogenised in water for 2 minutes in a Waring blender operating at high speed. The homogenate was washed and filtered (initially through 2mm, then through 1mm filters) using about 4 litres of water per 100gms of tubers (6 extractions). Washed starch granules were finally extracted with acetone and air dried.

Starch extracted from singly transformed potato plants (class A/SBE II antisense, or class B/SBE I antisense), or from double transformants (class A/SBE II and class B/SBE I antisense), or from untransformed control plants, was partially characterised. The results are shown in Table 1. The table shows the amount of SBE activity (units/gram tissue) in tubers from each transformed plant. The endotherm peak temperature (°C) of starch extracted from several plants was determined by DSC, and the onset temperature (°C) of pasting was determined by reference to a viscoamylograph ("RVA"), as described in WO 95/26407. The viscoamylograph profile was as follows: step 1 - 50°C for 2 minutes; step 2 - increase in temperature from 50°C to 95°C at a rate of 1.5°C per minute; step 3 - holding at 95°C for 15 minutes; step 4 - cooling from 95°C to 50°C at a rate of 1.5°C per minute; and finally, step 5 - holding at 50°C for 15 minutes. Table 1 shows the peak, pasting and set-back viscosities in stirring number units (SNUs), which is a measure of the amount of torque required to stir the suspensions. Peak viscosity may be defined for present purposes as the maximun viscosity attained during the heating phase (step 2) or the holding phase (step 3) of the viscoamylograph. Pasting viscosity may be defined as the viscosity attained by the starch suspensions at the end of the holding phase (step 3) of the viscoamylograph. Set-back viscosity may be defined as the viscosity of the starch suspension at the end of step 5 of the viscoamylograph.

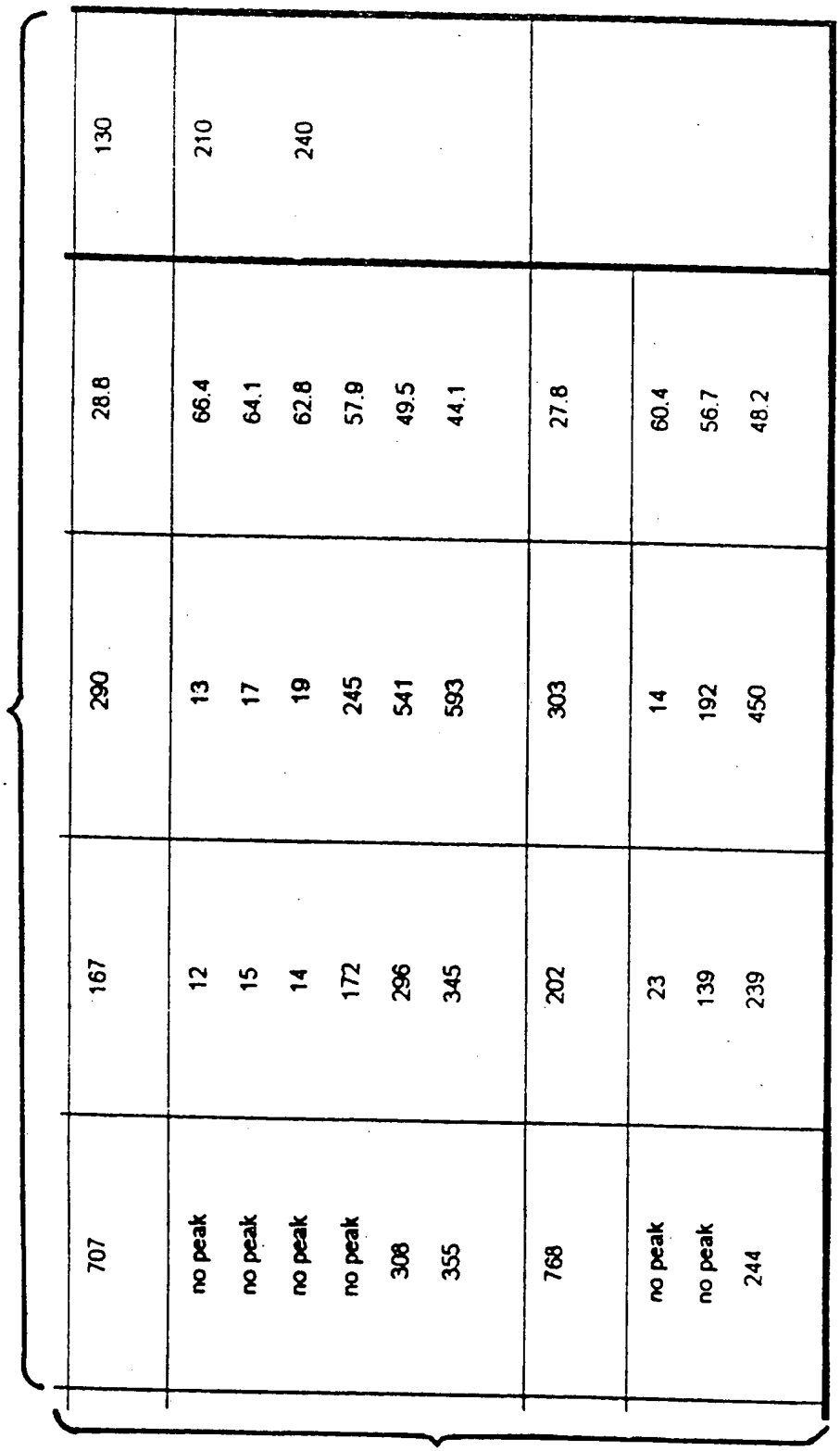
A determination of apparent amylose content (% w/w) was also performed, using the iodometric assay method of Morrison & Laignelet (1983 J. Cereal Sci. *1*, 9-20). The

Table 1

Sample description	Sample number	Tuber SBE activity (U/g starch)	DSC Peak temperature (°C)	Viscoamylograph (RVA) Onset temperature (°C)	Peak viscosity (SNU)	Pasting viscosity (SNU)	Set-back viscosity (SNU)	Apparent amylose content (% w/w)	Phosphorus content (mg/100g)
Untransformed control	148	7.6	65.8	65.5	545	181	260	31.2	68
	243	22.2	nd	62.6	761	135	241	29.1	
AS-Class A SBE	152	12.7	69.5	70.9	467	380	529	37.5	69
	249	13.9	nd	70.0	497	434	518	36.5	
AS-Class B SBE (17) (control)	145	0.7	66.9	66.6	669	177	305	29.8	111
AS-Class B SBE (17) + AS-Class A SBE	150	0.6	74.0	66.0	214	214	303	53.1	198
	181	0.5	73.0	76.6	349	324	618	40.9	206
AS-Class B SBE (16) (control)	144	1.6	64.5	64.7	714	154	258	29.0	97
AS-Class B SBE (16) + AS-Class A SBE	149	3.0	66.5	69.9	474	267	482	35.6	127
AS-Class B SBE (15) (control)	172	0.22	nd	65.4	707	167	280	28.6	130
AS-Class B SBE (15) + AS-Class A SBE	201	0.10	nd	>95	no peak	12	13	66.4	210
	208a	0.10	nd	>95	no peak	15	17	64.1	
	208	0.30	72.6-80.5	>95	no peak	14	19	62.8	240
	202	0.02	nd	69.4	no peak	172	245	57.9	
	212	1.40	nd	78.0	308	298	541	49.5	
	220	1.40	nd	75.6	355	345	593	44.1	
AS-Class B SBE (12) (control)	170	0.2	nd	66.5	768	202	303	27.6	
AS-Class B SBE (12) + AS-Class A SBE	238	0.7	nd	95.0	no peak	23	14	60.4	
	238a	0.9	nd	91.2	no peak	139	192	56.7	
	230a	0.8	nd	77.6	244	239	450	46.2	

RVA profile	50°C (2 min), 50-95°C (1.5°C/min), 95°C (15 min), 95-50°C (1.5°C/min), 50°C (15 min)
Pasting viscosity (47 min)	at end of 50°C (2min), 50-95°C (1.5°C/min), 95°C (15 min)
Set-back viscosity (92 min)	at end of profile
SBE	Starch Branching Enzyme
SNU	Instrument "Stirring Number Units" (arbitrary units)
nd	not determined

Viscoamylograph (RVA)			Apparent amylose content (% w/w)	Phosphorus content (mg/100g)
Peak viscosity (SNU)	Pasting viscosity (SNU)	Set-back viscosity (SNU)		
545	161	280	31.2	68
761	135	241	29.1	
467	360	529	37.5	89
497	434	518	38.5	
669	177	305	29.8	111
214	214	303	53.1	198
349	324	618	40.9	206
714	154	258	29.0	97
474	267	482	35.6	127



707	167	290	28.8	130
no peak	12	13	66.4	210
no peak	15	17	64.1	
no peak	14	19	62.8	240
no peak	172	245	57.9	
308	296	541	49.5	
355	345	593	44.1	
768	202	303	27.8	
no peak	23	14	60.4	
no peak	139	192	56.7	
244	239	450	48.2	

149 (35.6% amylose): shaded triangle, pointing upwards - plant 152 (37.5%); shaded triangle, pointing downwards - plant 161 (40.9%); shaded diamond - plant 150 (53.1%); unshaded box - plant 236a (56.7%); unshaded circle - plant 236 (60.4%); unshaded triangle, pointing upwards - plant 201 (66.4%); unshaded triangle, pointing downwards - Hylon V starch, from maize (44.9 % amylose). The thin line denotes the heating profile.

With increasing amylose content, peak viscosities during processing to 95°C decrease, and the drop in viscosity from the peak until the end of the holding period at 95°C also generally decreases (indeed, for some of the starch samples there is an increase in viscosity during this period). Both of these results are indicative of reduced granule fragmentation, and hence *increased* granule stability during pasting. This property has not previously been available in potato starch without extensive prior chemical or physical modification. For applications where a maximal viscosity after processing to 95°C is desirable (i.e. corresponding to the viscosity after 47 minutes in the viscoamylograph test), starch from plant #152 would be selected as starches with both lower (Controls, #149) and higher (#161, #150) amylose contents have lower viscosities following this gelatinisation and pasting regime (Figure 13 and Table 1). It is believed that the viscosity at this stage is determined by a combination of the extent of granule swelling and the resistance of swollen granules to mechanical fragmentation. For any desired viscosity behaviour, one skilled in the art would select a potato starch from a range containing different amylose contents produced according to the invention by performing suitable standard viscosity tests.

Upon cooling pastes from 95°C to 50°C, potato starches from most plants transformed in accordance with the invention showed an increase in viscoamylograph viscosity as expected for partial reassociation of amylose. Starches from plants #149, 152 and 161 all show viscosities at 50°C significantly in excess of those for starches from control plants (Figure 13 and Table 1). This contrasts with the effect of elevated amylose contents in starches from maize plants (Figure 2) which show very low viscosities throughout the viscoamylograph test. Of particular note is the fact that, for similar amylose contents, starch from potato plant 150 (53% amylose) shows markedly increased viscosity compared with Hylon 5 starch (44.9% amylose) as illustrated in Figure 13. This demonstrates that

201, 208 and 208a did not display a clearly defined pasting viscosity peak during the heating phase. Microscopic analysis confirmed that the starch granule structure underwent only minor swelling during the experimental heating process. This property may well be particularly useful in certain applications, as will be apparent to those skilled in the art.

Some re-grown plants have so far been found to increase still further the apparent amylose content of starch extracted therefrom. Such increases may be due to:-

i) Growth and development of the first generation transformed plants may have been affected to some degree by the exogenous growth hormones present in the tissue culture system, which exogenoous hormones were not present during growth of the second generation plants; and

ii) Subsequent generations were grown under field conditions, which may allow for attainment of greater maturity than growth under laboratory conditions, it being generally held that amylose content of potato starch increases with maturity of the potato tuber.

Accordingly, it should be possible to obtain potato plants giving rise to tubers with starch having an amylose content in excess of the 66% level so far attained, simply by analysing a greater number of transformed plants and/or by re-growing transgenic plants through one or more generations under field conditions.

Table 1 shows that another characteristic of starch which is affected by the presence of anti-sense sequences to SBE is the phosphorus content. Starch from untransformed control plants had a phosphorus content of about 60-70mg/100gram dry weight (as determined according to the AOAC Official Methods of Analysis, 15th Edition, Method 948.09 "Phosphorus in Flour"). Introduction into the plant of an anti-sense SBE B sequence was found to cause a modest increase (about two-fold) in phosphorus content, which is in agreement with the previous findings reported at scientific meetings. Similarly, anti-sense to SBE A alone causes only a small rise in phosphorus content relative to untransformed controls. However, use of anti-sense to both SBE A and B in combination results in up to a four-fold increase in phosphorus content. which is far greater than any *in planta* phosphorus content previously demonstrated for potato starch.

This is useful in that. for certain applications. starch must be phosphorylated *in vitro* by

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: National Starch and Chemical Investment Holding Corporation
(B) STREET: 501 Silverside Road, Suite 27
(C) CITY: Wilmington
(D) STATE: Delaware
(E) COUNTRY: United States of America
(F) POSTAL CODE (ZIP): 19809

(ii) TITLE OF INVENTION: Improvements in or Relating to Plant Starch Composition

(iii) NUMBER OF SEQUENCES: 20

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

AAGGATCCGT CGACATCGAT AATACGACTC ACTATAGGGA TTTTTTTTTT TTTTTTT 57

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

AAGGATCCGT CGACATC 17

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

AGAACAACAA TTCCTAGCTC 20

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GGGGCCTTGA ACTCAGCAAT 20

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CGTCCCAGCA TTCGACATAA 20

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CTTGGATCCT TGAACTCAGC AATTTG 26

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

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ACGGCACAGA TAGTTGTTAC TTTCACTCTG GAGCTCGTGG TTATCATTGG ATGTGGGATT 1560

TCCGCCTCTT TAACTATGGA AACTGGGAGG TACTTAGGTA TCTTCTCTCA AATGCGAGAT 1620

GGTGGTTGGA TGAGTTCAAA TTTGATGGAT TTAGATTTGA TGGTGTGACA TCAATGATGT 1680

GTACTCACCA CGGATTATCG GTGGGATTCA CTGGGAACTA CGAGGAATAC TTTGGACTCG 1740

CAACTGATGT GGATGCTGTT GTGTATCTGA TGCTGGTCAA CGATCTTATT CATGGGCTTT 1800

TCCCAGATGC AATTACCATT GGTGAAGATG TTAGCGGAAT GCCGACATTT TGTGTTCCCG 1860

TTCAAGATGG GGGTGTTGGC TTTGACTATC GGCTGCATAT GGCAATTGCT GATAAATGGA 1920

TTGAGTTGCT CAAGAAACGG GATGAGGATT GGAGAGTGGG TGATATTGTT CATACACTGA 1980

CAAATAGAAG ATGGTCGGAA AAGTGTGTTT CATACGCTGA AAGTCATGAT CAAGCTCTAG 2040

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TGGATAGACC GTCAACATCA TTAATAGATC GTGGGATAGC ATTACACAAG ATGATTAGGC 2160

TTGTAACTAT GGGATTAGGA GGAGAAGGGT ACCTAAATTT CATGGGAAAT GAATTCGGCC 2220

ACCCTGAGTG GATTGATTTC CCTAGGGCTG AACAACACCT CTCTGATGGC TCAGTAATTC 2280

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ATGAGTTTAT GACTTCAGAA CACCAGTTCA TATCACGAAA GGATGAAGGA GATAGGATGA 2460

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CAAAAACATA TGTAAAATCG ATGAATTTAT GTCGAATGCT GGGACGATCG AATTCCTGCA 3000

GCC 3003

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TCATGGACAT CGTTCACAGC CATGCATCAA ATAATACTTT AGATGGACTG AACATGTTTG 1500

ACGGCACCGA TAGTTGTTAC TTTCACTCTG GAGCTCGTGG TTATCATTGG ATGTGGGATT 1560

CCGCCTCTTT AACTATGGAA ACTGGGAGGT ACTTAGGTAT CTTCTCTCAA ATGCGAGATG 1620

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TACTCACCAC GGATTATCGG TGGGATTCAC TGGGAACTAC GAGGAATACT TTGGACTCGC 1740

AACTGATGTG GATGCTGTTG TGTATCTGAT GCTGGTCAAC GATCTTATTC ATAGGCTTTT 1800

CCCAGATGCA ATTACCATTG GTGAAGATGT TAGCGGAATG CCGACATTTT GTATTCCCGT 1860

TCAAGATGGG GGTGTTGGCT TTGACTATCG GCTGCATATG GCAATTGCTG ATAAATGGAT 1920

TGAGTTGCTC AAGAAACGGG ATGAGGATTG GAGAGTGGGT GATATTGTTC ATACACTGAC 1980

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CGGTGATAAA ACTATAGCAT TCTGGCTGAT GGACAAGGAT ATGTATGATT TTATGGCTCT 2100

GGATAGACCG CCAACATCAT TAATAGATCG TGGGATAGCA TTGCACAAGA TGATTAGGCT 2160

TGTAACTATG GGATTAGGAG GAGAAGGGTA CCTAAATTTC ATGGGAAATG AATTCGGCCA 2220

CCCTGAGTGG ATTGATTTCC CTAGGGCTGA GCCACACCTT TCTGATGGCT CAGTAATTCC 2280

CGGAAACCAA TTCAGTTATG ATAAATGCAG ACGGAGATTT GACCTGGGAG ATGCAGAATA 2340

TTTAAGATAC CATGGGTTAC AAGAATTTGA CTGGGCTATG CAGTATCTTG AAGATAAATA 2400

TGAGTTTATG ACTTCAGAAC ACCAGTTCAT ATCACGAAAG GATGAAGGAG ATAGGATGAT 2460

TGTATTTGAA AGAGGAAACC TAGTTTTCGT CTTTAATTTT CACTGGACAA ATAGCTATTC 2520

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ATCGTATGAT GATCGTCCTT GTTCAATTAT GGTGTATGCA CCTAGTAGAA CAGCAGTGGT 2700

CTATGCACTA GTAGACAAAC TAGAAGTAGC AGTAGTAGAA GAACCCATTG AAGAATGAAC 2760

GAACTTGTGA TCGCGTTGAA AGATTTGAAC GTTACTTGGT CATCCACATA GAGCTTCTTG 2820

ACATCAGTCT TGGCGGAATT GCATGTGACA ACAAGGTTTG CAGTTCTTTC CACTATTAGT 2880

AGTCCACCGA TATACGCAGA GATGAAGTGC TGAACAAACA TATGTAAAAT CGATGAATTT 2940

ATGTCGAATG CTGGGACGAT CGAATTCCTG CAGCC 2975

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CTA CAA CTA CAA GAA GGT GGT AAA CTG GAG GAG TCT AAA ACA TTA AAT      603
Leu Gln Leu Gln Glu Gly Gly Lys Leu Glu Glu Ser Lys Thr Leu Asn
        140                 145                 150

ACT TCT GAA GAG ACA ATT ATT GAT GAA TCT GAT AGG ATC AGA GAG AGG      651
Thr Ser Glu Glu Thr Ile Ile Asp Glu Ser Asp Arg Ile Arg Glu Arg
    155                 160                 165

GGC ATC CCT CCA CCT GGA CTT GGT CAG AAG ATT TAT GAA ATA GAC CCC      699
Gly Ile Pro Pro Pro Gly Leu Gly Gln Lys Ile Tyr Glu Ile Asp Pro
170                 175                 180                 185

CTT TTG ACA AAC TAT CGT CAA CAC CTT GAT TAC AGG TAT TCA CAG TAC      747
Leu Leu Thr Asn Tyr Arg Gln His Leu Asp Tyr Arg Tyr Ser Gln Tyr
                190                 195                 200

AAG AAA CTG AGG GAG GCA ATT GAC AAG TAT GAG GGT GGT TTG GAA GCC      795
Lys Lys Leu Arg Glu Ala Ile Asp Lys Tyr Glu Gly Gly Leu Glu Ala
            205                 210                 215

TTT TCT CGT GGT TAT GAA AAA ATG GGT TTC ACT CGT AGT GCT ACA GGT      843
Phe Ser Arg Gly Tyr Glu Lys Met Gly Phe Thr Arg Ser Ala Thr Gly
        220                 225                 230

ATC ACT TAC CGT GAG TGG GCT CTT GGT GCC CAG TCA GCT GCC CTC ATT      891
Ile Thr Tyr Arg Glu Trp Ala Leu Gly Ala Gln Ser Ala Ala Leu Ile
    235                 240                 245

GGA GAT TTC AAC AAT TGG GAC GCA AAT GCT GAC ATT ATG ACT CGG AAT      939
Gly Asp Phe Asn Asn Trp Asp Ala Asn Ala Asp Ile Met Thr Arg Asn
250                 255                 260                 265

GAA TTT GGT GTC TGG GAG ATT TTT CTG CCA AAT AAT GTG GAT GGT TCT      987
Glu Phe Gly Val Trp Glu Ile Phe Leu Pro Asn Asn Val Asp Gly Ser
                270                 275                 280

CCT GCA ATT CCT CAT GGG TCC AGA GTG AAG ATA CGT ATG GAC ACT CCA     1035
Pro Ala Ile Pro His Gly Ser Arg Val Lys Ile Arg Met Asp Thr Pro
            285                 290                 295

TCA GGT GTT AAG GAT TCC ATT CCT GCT TGG ATC AAC TAC TCT TTA CAG     1083
Ser Gly Val Lys Asp Ser Ile Pro Ala Trp Ile Asn Tyr Ser Leu Gln
        300                 305                 310

CTT CCT GAT GAA ATT CCA TAT AAT GGA ATA CAT TAT GAT CCA CCC GAA     1131
Leu Pro Asp Glu Ile Pro Tyr Asn Gly Ile His Tyr Asp Pro Pro Glu
    315                 320                 325

GAG GAG AGG TAT ATC TTC CAA CAC CCA CGG CCA AAG AAA CCA AAG TCG     1179
Glu Glu Arg Tyr Ile Phe Gln His Pro Arg Pro Lys Lys Pro Lys Ser
330                 335                 340                 345

CTG AGA ATA TAT GAA TCT CAT ATT GGA ATG AGT AGT CCG GAG CCT AAA     1227
Leu Arg Ile Tyr Glu Ser His Ile Gly Met Ser Ser Pro Glu Pro Lys
                350                 355                 360
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ATG GCA ATT GCT GAT AAA CGG ATT GAG TTG CTC AAG AAA CGG GAT GAG          1947
Met Ala Ile Ala Asp Lys Arg Ile Glu Leu Leu Lys Lys Arg Asp Glu
                590                 595                 600

GAT TGG AGA GTG GGT GAT ATT GTT CAT ACA CTG ACA AAT AGA AGA TGG          1995
Asp Trp Arg Val Gly Asp Ile Val His Thr Leu Thr Asn Arg Arg Trp
            605                 610                 615

TCG GAA AAG TGT GTT TCA TAC GCT GAA AGT CAT GAT CAA GCT CTA GTC          2043
Ser Glu Lys Cys Val Ser Tyr Ala Glu Ser His Asp Gln Ala Leu Val
        620                 625                 630

GGT GAT AAA ACT ATA GCA TTC TGG CTG ATG GAC AAG GAT ATG TAT GAT          2091
Gly Asp Lys Thr Ile Ala Phe Trp Leu Met Asp Lys Asp Met Tyr Asp
    635                 640                 645

TTT ATG GCT CTG GAT AGA CCG TCA ACA TCA TTA ATA GAT CGT GGG ATA          2139
Phe Met Ala Leu Asp Arg Pro Ser Thr Ser Leu Ile Asp Arg Gly Ile
650                 655                 660                 665

GCA TTG CAC AAG ATG ATT AGG CTT GTA ACT ATG GGA TTA GGA GGA GAA          2187
Ala Leu His Lys Met Ile Arg Leu Val Thr Met Gly Leu Gly Gly Glu
                670                 675                 680

GGG TAC CTA AAT TTC ATG GGA AAT GAA TTC GGC CAC CCT GAG TGG ATT          2235
Gly Tyr Leu Asn Phe Met Gly Asn Glu Phe Gly His Pro Glu Trp Ile
            685                 690                 695

GAT TTC CCT AGG GCT GAA CAA CAC CTC TCT GAT GGC TCA GTA ATC CCC          2283
Asp Phe Pro Arg Ala Glu Gln His Leu Ser Asp Gly Ser Val Ile Pro
        700                 705                 710

GGA AAC CAA TTC AGT TAT GAT AAA TGC AGA CGG AGA TTT GAC CTG GGA          2331
Gly Asn Gln Phe Ser Tyr Asp Lys Cys Arg Arg Arg Phe Asp Leu Gly
    715                 720                 725

GAT GCA GAA TAT TTA AGA TAC CGT GGG TTG CAA GAA TTT GAC CGG CCT          2379
Asp Ala Glu Tyr Leu Arg Tyr Arg Gly Leu Gln Glu Phe Asp Arg Pro
730                 735                 740                 745

ATG CAG TAT CTT GAA GAT AAA TAT GAG TTT ATG ACT TCA GAA CAC CAG          2427
Met Gln Tyr Leu Glu Asp Lys Tyr Glu Phe Met Thr Ser Glu His Gln
                750                 755                 760

TTC ATA TCA CGA AAG GAT GAA GGA GAT AGG ATG ATT GTA TTT GAA AAA          2475
Phe Ile Ser Arg Lys Asp Glu Gly Asp Arg Met Ile Val Phe Glu Lys
            765                 770                 775

GGA AAC CTA GTT TTT GTC TTT AAT TTT CAC TGG ACA AAA AGC TAT TCA          2523
Gly Asn Leu Val Phe Val Phe Asn Phe His Trp Thr Lys Ser Tyr Ser
        780                 785                 790

GAC TAT CGC ATA GCC TGC CTG AAG CCT GGA AAA TAC AAG GTT GCC TTG          2571
Asp Tyr Arg Ile Ala Cys Leu Lys Pro Gly Lys Tyr Lys Val Ala Leu
    795                 800                 805
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Ala Ser Thr Asp Val Asp Ser Ser Thr Met Glu His Ala Ser Gln Ile
100 105 110

Lys Thr Glu Asn Asp Asp Val Glu Pro Ser Ser Asp Leu Thr Gly Ser
115 120 125

Val Glu Glu Leu Asp Phe Ala Ser Ser Leu Gln Leu Gln Glu Gly Gly
130 135 140

Lys Leu Glu Glu Ser Lys Thr Leu Asn Thr Ser Glu Glu Thr Ile Ile
145 150 155 160

Asp Glu Ser Asp Arg Ile Arg Glu Arg Gly Ile Pro Pro Pro Gly Leu
165 170 175

Gly Gln Lys Ile Tyr Glu Ile Asp Pro Leu Leu Thr Asn Tyr Arg Gln
180 185 190

His Leu Asp Tyr Arg Tyr Ser Gln Tyr Lys Lys Leu Arg Glu Ala Ile
195 200 205

Asp Lys Tyr Glu Gly Gly Leu Glu Ala Phe Ser Arg Gly Tyr Glu Lys
210 215 220

Met Gly Phe Thr Arg Ser Ala Thr Gly Ile Thr Tyr Arg Glu Trp Ala
225 230 235 240

Leu Gly Ala Gln Ser Ala Ala Leu Ile Gly Asp Phe Asn Asn Trp Asp
245 250 255

Ala Asn Ala Asp Ile Met Thr Arg Asn Glu Phe Gly Val Trp Glu Ile
260 265 270

Phe Leu Pro Asn Asn Val Asp Gly Ser Pro Ala Ile Pro His Gly Ser
275 280 285

Arg Val Lys Ile Arg Met Asp Thr Pro Ser Gly Val Lys Asp Ser Ile
290 295 300

Pro Ala Trp Ile Asn Tyr Ser Leu Gln Leu Pro Asp Glu Ile Pro Tyr
305 310 315 320

Asn Gly Ile His Tyr Asp Pro Pro Glu Glu Glu Arg Tyr Ile Phe Gln
325 330 335

His Pro Arg Pro Lys Lys Pro Lys Ser Leu Arg Ile Tyr Glu Ser His
340 345 350

Ile Gly Met Ser Ser Pro Glu Pro Lys Ile Asn Ser Tyr Val Asn Phe
355 360 365

Arg Asp Glu Val Leu Pro Arg Ile Lys Lys Leu Gly Tyr Asn Ala Leu
370 375 380

Gln Ile Met Ala Ile Gln Glu His Ser Tyr Tyr Ala Ser Phe Gly Tyr
385 390 395 400

His Leu Ser Asp Gly Ser Val Ile Pro Gly Asn Gln Phe Ser Tyr Asp
705 710 715 720

Lys Cys Arg Arg Arg Phe Asp Leu Gly Asp Ala Glu Tyr Leu Arg Tyr
725 730 735

Arg Gly Leu Gln Glu Phe Asp Arg Pro Met Gln Tyr Leu Glu Asp Lys
740 745 750

Tyr Glu Phe Met Thr Ser Glu His Gln Phe Ile Ser Arg Lys Asp Glu
755 760 765

Gly Asp Arg Met Ile Val Phe Glu Lys Gly Asn Leu Val Phe Val Phe
770 775 780

Asn Phe His Trp Thr Lys Ser Tyr Ser Asp Tyr Arg Ile Ala Cys Leu
785 790 795 800

Lys Pro Gly Lys Tyr Lys Val Ala Leu Asp Ser Asp Asp Pro Leu Phe
805 810 815

Gly Gly Phe Gly Arg Ile Asp His Asn Ala Glu Tyr Phe Thr Phe Glu
820 825 830

Gly Trp Tyr Asp Asp Arg Pro Arg Ser Ile Met Val Tyr Ala Pro Cys
835 840 845

Lys Thr Ala Val Val Tyr Ala Leu Val Asp Lys Glu Glu Glu Glu Glu
850 855 860

Glu Glu Glu Glu Glu Glu Val Ala Ala Val Glu Glu Val Val Val Glu
865 870 875 880

Glu Glu

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2576 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

TCATTAAAGA GGAGAAATTA ACTATGAGAG GATCTCACCA TCACCATCAC CATGGGATCT 60

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AAGTCCTTGT GCCTGGAACC CAGAGTGATA GCTCCTCATC CTCAACAAAC CAATTTGAGT 180

TCACTGAGAC ATCTCCAGAA AATTCCCCAG CATCAACTGA TGTAGATAGT TCAACAATGG 240

AACACGCTAG CCAGATTAAA ACTGAGAACG ATGACGTTGA GCCGTCAAGT GATCTTACAG 300

GGAAATGAAT TCGGCCACCC TGAGTGGATT GATTTCCCTA GGGCTGAACA ACACCTCTCT 2040

GATGACTCAG TAATTCCCGG AAACCAATTC AGTTATGATA AATGCAGACG GAGATTTGAC 2100

CTGGGAGATG CAGAATATTT AAGATACCGT GGGTTGCAAG AATTTGACCG GGCTATGCAG 2160

TATCTTGAAG ATAAATATGA GTTTATGACT TCAGAACACC AGTTCATATC ACGAAAGGAT 2220

GAAGGAGATA GGATGATTGT ATTTGAAAAA GGAAACCTAG TTTTTGTCTT TAATTTTCAC 2280

TGGACAAAAA GCTATTCAGA CTATCGCATA GGCTGCCTGA AGCCTGGAAA ATACAAGGTT 2340

GCCTTGGACT CAGATGATCC ACTTTTTGGT GGCTTCGGGA GAATTGATCA TAATGCCGAA 2400

TATTTCACCT TTGAAGGATG GTATGATGAT CGTCCTCGTT CAATTATGGT GTATGCACCT 2460

TGTAGAACAG CAGTGGTCTA TGCACTAGTA GACAAAGAAG AAGAAGAAGA AGAAGAAGAA 2520

GAAGAAGTAG CAGTAGTAGA AGAAGTAGTA GTAGAAGAAG AATGAACGAA CTTGTG 2576

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2529 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

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AAAGTCTTCT TACAATTCCG AATCCCGACC TTCTACAGTT GCAGCATCGG GGAAAGTCCT 120

TGTGCCTGGA AYCCAGAGTG ATAGCTCCTC ATCCTCAACA GACCAATTTG AGTTCACTGA 180

GACATCTCCA GAAAATTCCC CAGCATCAAC TGATGTAGAT AGTTCAACAA TGGAACACGC 240

TAGCCAGATT AAAACTGAGA ACGATGACGT TGAGCCGTCA AGTGATCTTA CAGGAAGTGT 300

TGAAGAGCTG GATTTTGCTT CATCACTACA ACTACAAGAA GGTGGTAAAC TGGAGGAGTC 360

TAAAACATTA AATACTTCTG AAGAGACAAT TATTGATGAA TCTGATAGGA TCAGAGAGAG 420

GGGCATCCCT CCACCTGGAC TTGGTCAGAA GATTTATGAA ATAGACCCCC TTTTGACAAA 480

CTATCGTCAA CACCTTGATT ACAGGTATTC ACAGTACAAG AAACTGAGGG AGGCAATTGA 540

CAAGTATGAG GGTGGTTTGG AAGCTTTTTC TCGTGGTTAT GAAAAAATGG GTTTCACTCG 600

TAGTGCTACA GGTATCACTT ACCGTGAGTG GGCTCCTGGT GCCCAGTCAG CTGCCCTCAT 660

TGGAGATTTC AACAATTGGG ACGCAAATGC TGACATTATG ACTCGGAATG AATTTGGTGT 720

CTGGGAGATT TTTCTGCCAA ATAATGTGGA TGGTTCTCCT GCAATTCCTC ATGGGTCCAG 780

AACAGCAGTG GTCTATGCAC TAGTAGACAA ANTAGAAGNA GAAGAAGAAG AAGAANCCGN 2520

NGAAGAATT 2529

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3231 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

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ACACTCAGTT AGTTACACTC CTATCACTCA TCAGATCTCT ATTTTTTCTC TTAATTCCAA 180

CCAAGGAATG AATTAAAAGA TTAGATTTGA AGGAGAGAAG AAGAAAGATG GTGTATACAC 240

TCTCTGGAGT TCGTTTTCCT ACTGTTCCAT CAGTGTACAA ATCTAATGGA TTCAGCAGTA 300

ATGGTGATCG GAGGAATGCT AATGTTTCTG TATTCTTGAA AAAGCACTCT CTTTCACGGA 360

AGATCTTGGC TGAAAAGTCT TCTTACGATT CCGAATCCCG ACCTTCTACA GTTGCAGCAT 420

CGGGGAAAGT CCTTGTACCT GGAATCCAGA GTGATAGCTC CTCATCCTCA ACAGACCAAT 480

TTGAGTTCAC TGAGACAGCT CCAGAAAATT CCCCAGCATC AACTGATGTG GATAGTTCAA 540

CAATGGAACA CGCTAGCCAG ATTAAAACTG AGAACGATGA CGTTGAGCCG TCAAGTGATC 600

TTACAGGAAG TGTTGAAGAG TTGGATTTTG CTTCATCACT ACAACTACAA GAAGGTGGTA 660

AACTGGAGGA GTCTAAAACA TTAAATACTT CTGAAGAGAC AATTATTGAT GAATCTGATA 720

GGATCAGAGA GAGGGGCATC CCTCCACCTG GACTTGGTCA GAAGATTTAT GAAATAGACC 780

CCCTTTTGAC AAACTATCGT CAACACCTTG ATTACAGGTA TTCACAGTAC AAGAAAATGA 840

GGGAGGCAAT TGACAAGTAT GAGGGTGGTT TGGAAGCTTT TTCTCGTGGT TATGAAAAAA 900

TGGGTTTCAC TCGTAGTGCT ACAGGTATCA CTTACCGTGA GTGGGCTCCT GGTGCCCAGT 960

CAGCTGCTCT CATTGGAGAT TTCAACAATT GGGACGCAAA TGCTGACATT ATGACTCGGA 1020

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CTCATGGGTC CAGAGTGAAG ATACGCATGG ACACTTCATC AGGTGTTAAG GATTCCATTC 1140

CTGCTTGGAT CAACTACTCT TTACAGCTTC CTGATGAAAT TCCATATAAT GGAATATATT 1200

ATGATCCACC CGAAGAGGAG AGGTATGTCT TCCAACACCC ACGGCCAAAG AAACCAAAGT 1260

GCGGAATTTC ATGTGACAAA AGGTTTGCAA TTCTTTCCAC TATTAGTAGT GCAACGATAT 3000

ACGCAGAGAT GAAGTGCTGA ACAAACATAT GTAAAATCGA TGAATTTATG TCGAATGCTG 3060

GGACGGGCTT CAGCAGGTTT TGCTTAGTGA GTTCTGTAAA TTGTCATCTC TTTANATGTA 3120

CAGCCCACTA GAAATCAATT ATGTGAGACC TAAAAAACAA TAACCATAAA ATGGAAATAG 3180

TGCTGATCTA ATGATGTTTT AANCCNNNNA AAAAAAAAAA AAAAACTCGA G 3231

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2578 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

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AAGTCCTTGT GCCTGGAACC CAGAGTGATA GCTCCTCATC CTCAACAAAC CAATTTGAGT 180

TCACTGAGAC ATCTCCAGAA AATTCCCCAG CATCAACTGA TGTAGATAGT TCAACAATGG 240

AACACGCTAG CCAGATTAAA ACTGAGAACG ATGACGTTGA GCCGTCAAGT GATCTTACAG 300

GAAGTGTTGA AGAGCTGGAT TTTGCTTCAT CACTACAACT ACAAGAAGGT GGTAAACTGG 360

AGGAGTCTAA AACATTAAAT ACTTCTGAAG AGACAATTAT TGATGAATCT GATAGGATCA 420

GAGAGAGGGG CATCCCTCCA CCTGGACTTG GTCAGAAGAT TTATGAAATA GACCCCCTTT 480

TGACAAACTA TCGTCAACAC CTTGATTACA GGTATTCACA GTACAAGAAA CTGAGGGAGG 540

CAATTGACAA GTATGAGGGT GGTTTGGAAG CTTTTTCTCG TGGTTATGAA AAAATGGGTT 600

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CCCTCATTGG AGATTTCAAC AATTGGGACG CAAATGCTGA CATTATGACT CGGAATGAAT 720

TTGGTGTCTG GGAGATTTTT CTGCCAAATA ATGTGGATGG TTCTCCTGCA ATTCCTCATG 780

GGTCCAGAGT GAAGATACGT ATGGACACTC CATCAGGTGT TAAGGATTCC ATTCCTGCTT 840

GGATCAACTA CTCTTCACAG CTTCCTGATG AAATTCCATA TAATGGAATA TATTATGATC 900

CACCCGAAGA GGAGAGGTAT ATCTTCCAAC ACCCACGGCC AAAGAAACCA AAGTCGCTGA 960

GAATATATGA ATCTCATATT GGAATGAGTA GTCCGGAGCC TAAAATTAAC TCATACGTGA 1020

ATTTTAGAGA TGAAGTTCTT CCTCGCATAA AAAAGCTTGG GTACAATGCG GTGCAAATTA 1080

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

AATTTYATGG GNAAYGARTT YGG 23

9. Starch which as extracted from a potato plant by wet milling at ambient temperature has a pasting viscosity in the range 214 - 434 SNUs, as judged by viscoamylograph conducted according to the protocol defined in claim 7.

10. Starch which as extracted from a potato plant by wet milling at ambient temperature has a set-back viscosity in the range 450 - 618 SNUs, as judged by viscoamylograph conducted according to the protocol defined in claim 7.

11. Starch which as extracted from a potato plant by wet milling at ambient temperature has a set-back viscosity in the range 14 - 192 SNUs, as judged by viscoamylograph conducted according to the protocol defined in claim 7.

12. Starch which as extracted from a potato plant by wet milling at ambient temperature has a peak viscosity in the range 200 - 500 SNUs and a set-back viscosity in the range 275-618 SNUs as judged by viscoamylograph according to the protocol defined in claim 7.

13. Starch which as extracted from a potato plant by wet milling at ambient temperature has a viscosity which does not decrease between the start of the heating phase (step 2) and the start of the final holding phase (step 5) and has a set-back viscosity of 303 SNUs or less as judged by viscoamylograph according to the protocol defined in claim 7.

14. Starch which as extracted from a potato plant by wet milling at ambient temperature displays no significant increase in viscosity as judged by viscoamylograph conducted according to the protocol defined in claim 7.

15. Starch which as extracted from a potato plant by wet milling at ambient temperature, is in accordance with claim 7 and in accordance with any one of claims 8 to 14.

16. Starch according to any one of claims 7 to 15, having an amylose content in the range 35 - 66%, as judged by the method of Morrison & Laignelet defined in claim 1.

enzyme (SBE) obtainable from potato plants.

28. A nucleotide sequence according to claim 27, encoding a polypeptide comprising substantially the amino acid sequence of residues 49 to 882 of the sequence shown in Figure 5.

29. A nucleotide sequence according to claim 27 or 28, comprising substantially the sequence of nucleotides 289 to 2790 of the sequence shown in Figure 5, or a functional equivalent thereof.

30. A nucleotide sequence according to claim 29, further comprising the sequence of nucleotides 145 to 288 of the sequence shown in Figure 5, or a functional equivalent thereof.

31. A nucleotide sequence according to claim 27, comprising the sequence of nucleotides 228 to 2855 of the sequence labelled psbe2con.seq in Figure 8, or a functional equivalent thereof.

32. A nucleotide sequence according to claim 27, comprising the sequence of nucleotides 57 to 2564 of the sequence labelled as psbe2con.seq in Figure 12, or a functional equivalent thereof.

33. A nucleotide sequence according to any one of claims 27 to 32, comprising an in-frame ATG start codon, and optionally including a 5' and/or a 3' untranslated region.

34. A nucleotide sequence according to claim 27, comprising the sequence of nucleotides 45 to 3200 of the sequence labelled as psbe2con.seq in Figure 8, or a functional equivalent thereof.

35. A nucleic acid construct comprising a sequence in accordance with any one of claims 27 to 34.

introducing into the plant one or more further sequences.

46. A method according to claim 45, wherein one or more of the further sequences are operably linked in the anti-sense orientation to a suitable promoter active in the plant.

47. A method according to claim 45 or 46, wherein the further sequence comprises a portion of a class B SBE nucleotide sequence.

48. A method according to any one of claims 42 to 47, effective in altering the starch composition of a plant.

49. A plant or plant cell having characteristics altered by the method of any one of claims 42 to 48, or the progeny of such a plant, or part of such a plant.

50. A plant according to claim 49, selected from one of the following: potato, pea, tomato, maize, wheat, rice, barley, sweet potato, and cassava.

51. A tuber or other storage organ from a plant according to claim 49 or 50.

52. Use of a tuber or other storage organ according to claim 51, in the preparation and/or processing of a foodstuff.

53. A plant according to claim 49 or 50, containing starch which, as extracted from the plant by wet milling at ambient temperature, has an elevated viscosity onset temperature as judged by viscoamylograph conducted according to the protocol defined in claim 7, compared to starch extracted from a similar, but unaltered, plant.

54. A plant according to claim 53, wherein the viscosity onset temperature is elevated by an amount in the range of 10 to 25°C.

55. A plant according to claim 49 or 50, containing starch which, as extracted from the plant by wet milling at ambient temperature, has a decreased peak viscosity as judged by

63. A plant according to claim 49 or 50, containing starch which, as extracted from the plant, has a phosphorus content in excess of 200mg/100grams dry weight starch.

64. Starch obtainable from a plant according to any one of claims 49, 50 or 53 - 63.

65. Starch according to claim 64 and further in accordance with any one of claims 1 - 22.

66. A method of modifying starch *in vitro*, comprising treating starch under suitable conditions with an effective amount of a polypeptide in accordance with any one of claims 38 to 41.

67. A potato plant or part thereof which, in its wild type possesses an effective SBE A gene, but which plant has been altered such that there is no effective expression of an SBE A polypeptide within the cells of at least part of the plant.

68. A potato plant according to claim 67, wherein the alteration is effected by a method according to any one of claims 42-48.

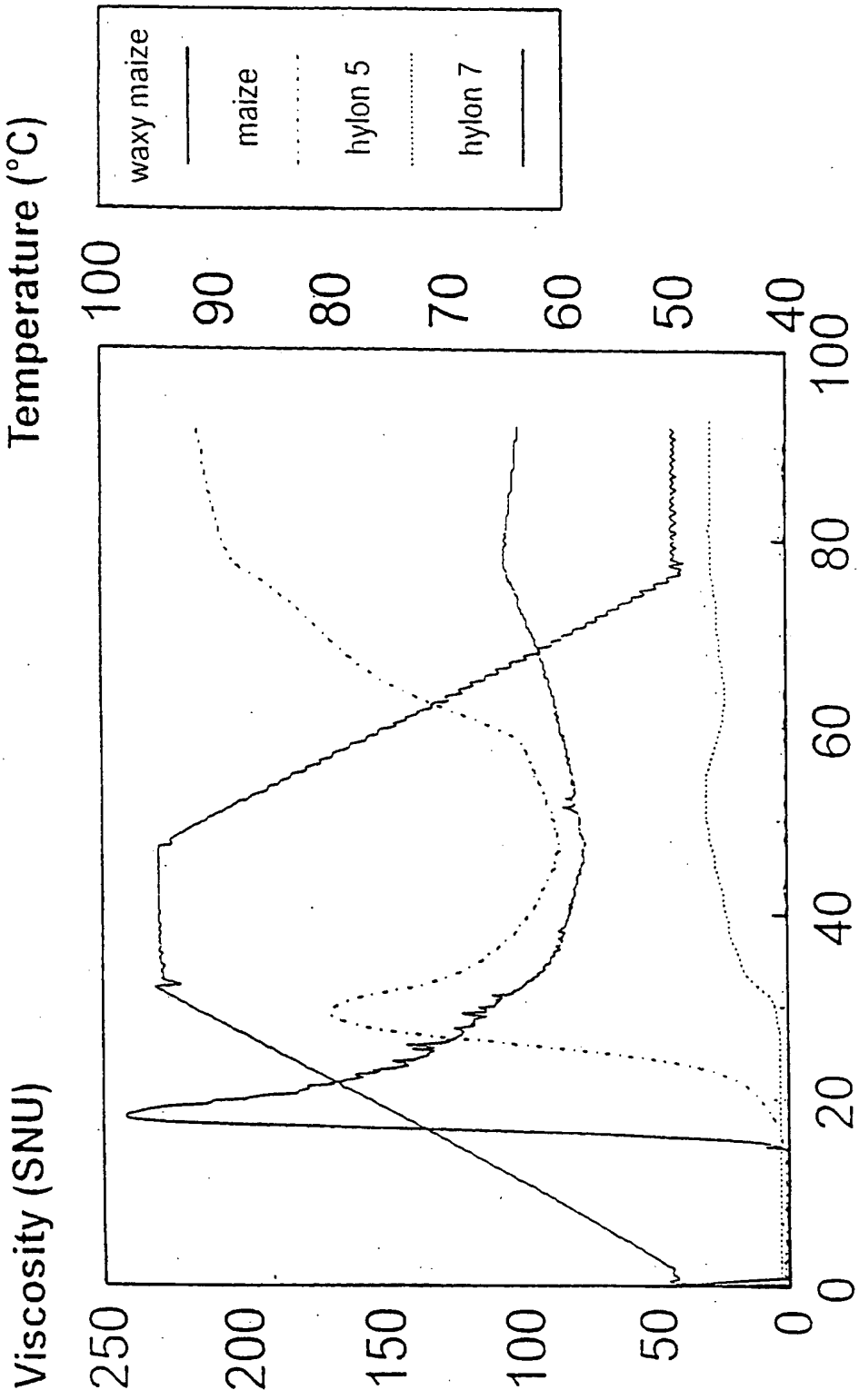

Fig. 2

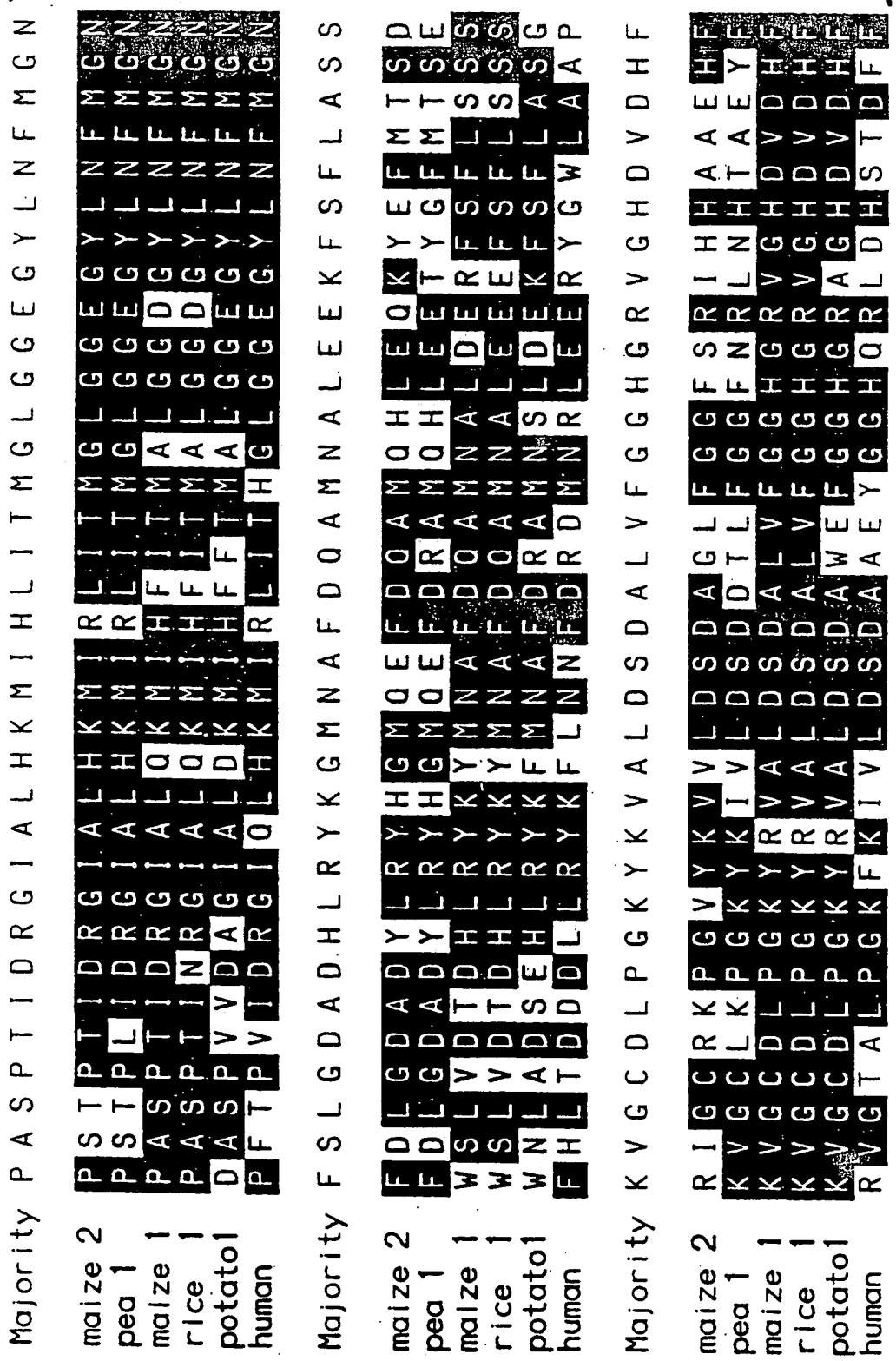

Fig. 4a SHEET 1

```
Majority  D E X X S E - R - - - - - - - - - - - - - - - - - - - - - - - - - - - -

maize 2   E .
pea 1     D G V E S E P I E L S
maize 1   D E A G A G R R L H A K A E T G K T S P A E S I D V K A S R A S S K
rice 1    D E D R E E L R R G G
potato1   D E R M S E T E D Y Q
human     D L P N .

          - - - - - - - - - - - - - - - - - - - - - - - - -

          E D K E A T A G G K K G W K F A R Q P S D Q D T K .      800
                                                                   856
                                                                   824
                                                                   769
                                                                   789
                                                                   703
```

Fig. 4a SHEET 3

```
TTGATGGGGCCTTGAACTCAGCAATTTGACACTCAGTTAGTTACA
AACTACCCCGGAACTTGAGTCGTTAAACTGTGAGTCAATCAATGT

AAGGAATGAATAAAAGGATAGATTTGTAAAAACCCTAAGGAGAGA
TTCCTTACTTATTTTCCTATCTAAACATTTTTGGGATTCCTCTCT
      M  N  K  R  I  D  L

GTTCCATCAGTGTACAAATCTAATGGATTCAGCAGTAATGGTGAT
CAAGGTAGTCACATGTTTAGATTACCTAAGTCGTCATTACCACTA
 V  P  S  V  Y  K  S  N  G  F  S  S  N  G  D

          Bgl II                          EcoR I
TCACGGAAGATCTTGGCTGAAAAGTCTTCTTACAATTCCGAATTC
AGTGCCTTCTAGAACCGACTTTTCAGAAGAATGTTAAGGCTTAAG
 S  R  K  I  L  A  E  K  S  S  Y  N  S  E  F

ACCCAGAGTGATAGCTCCTCATCCTCAACAGACCAATTTGAGTTC
TGGGTCTCACTATCGAGGAGTAGGAGTTGTCTGGTTAAACTCAAG
 T  Q  S  D  S  S  S  S  S  T  D  Q  F  E  F

AGTTCAACAATGGAACACGCTAGCCAGATTAAAACTGAGAACGAT
TCAAGTTGTTACCTTGTGCGATCGGTCTAATTTTGACTCTTGCTA
 S  S  T  M  E  H  A  S  Q  I  K  T  E  N  D

GATTTTGCTTCATCACTACAACTACAAGAAGGTGGTAAACTGGAG
CTAAAACGAAGTAGTGATGTTGATGTTCTTCCACCATTTGACCTC
 D  F  A  S  S  L  Q  L  Q  E  G  G  K  L  E
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Fig 5 Sheet 2

Fig. 5 SHEET 1

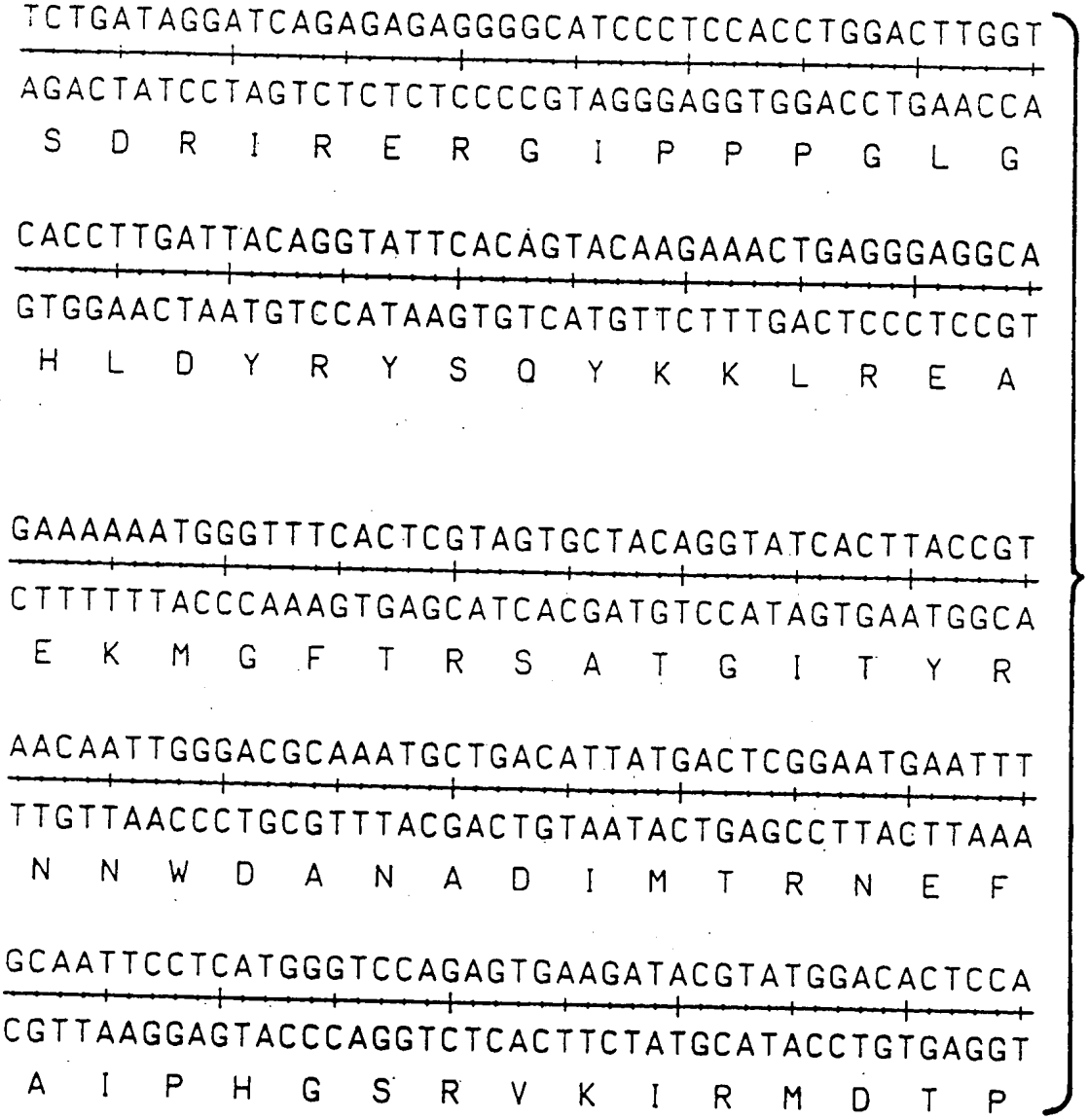

Fig.5 Sheet 4

Fig. 5 SHEET 3

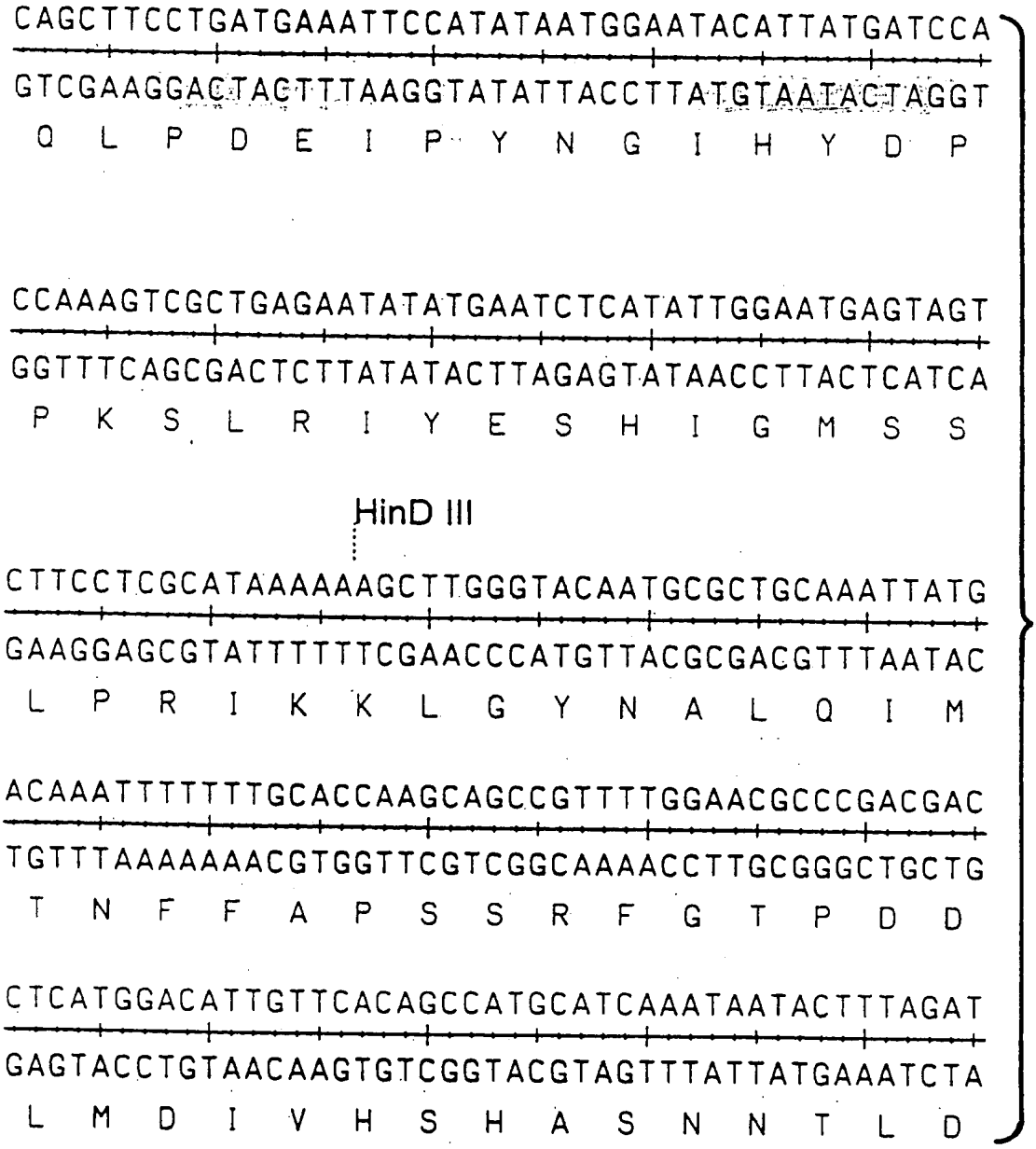

Fig.5 Sheet 6

Fig. 5 SHEET 5

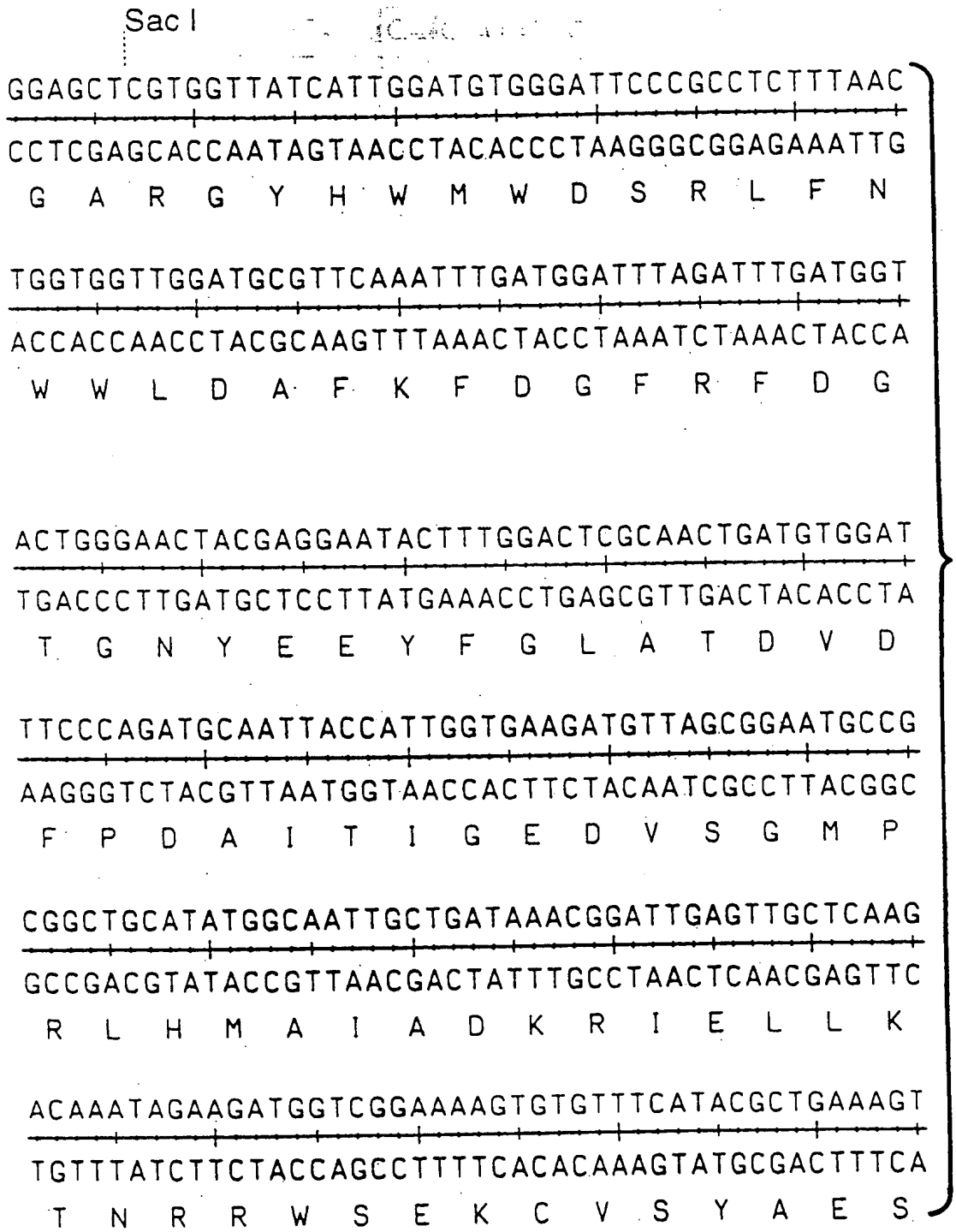

Fig 5 Sheet 8

Fig. 5 SHEET 7

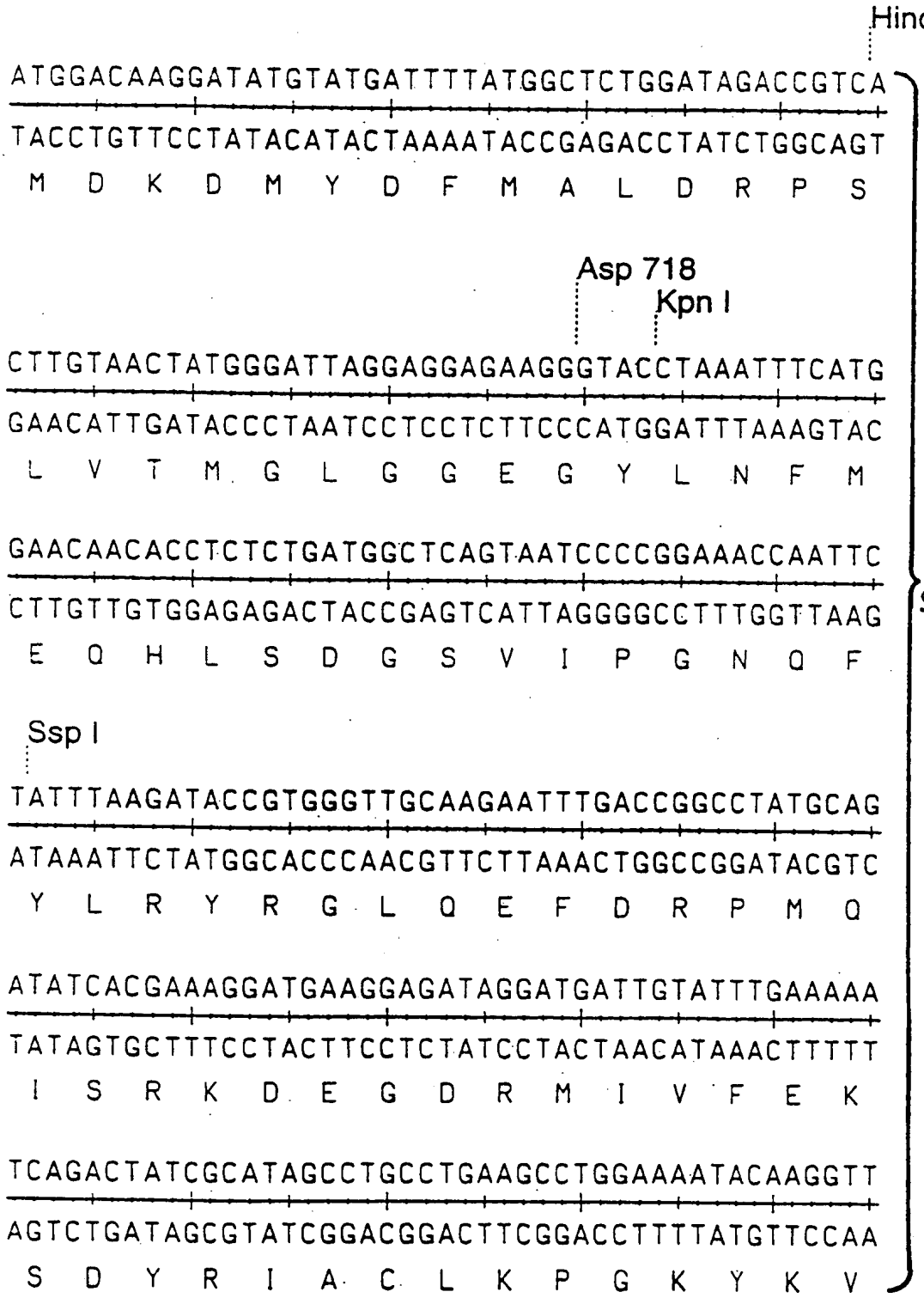

Fig.5 Sheet 10

Fig. 5 SHEET 9

SUBSTITUTE SHEET (RULE 26)

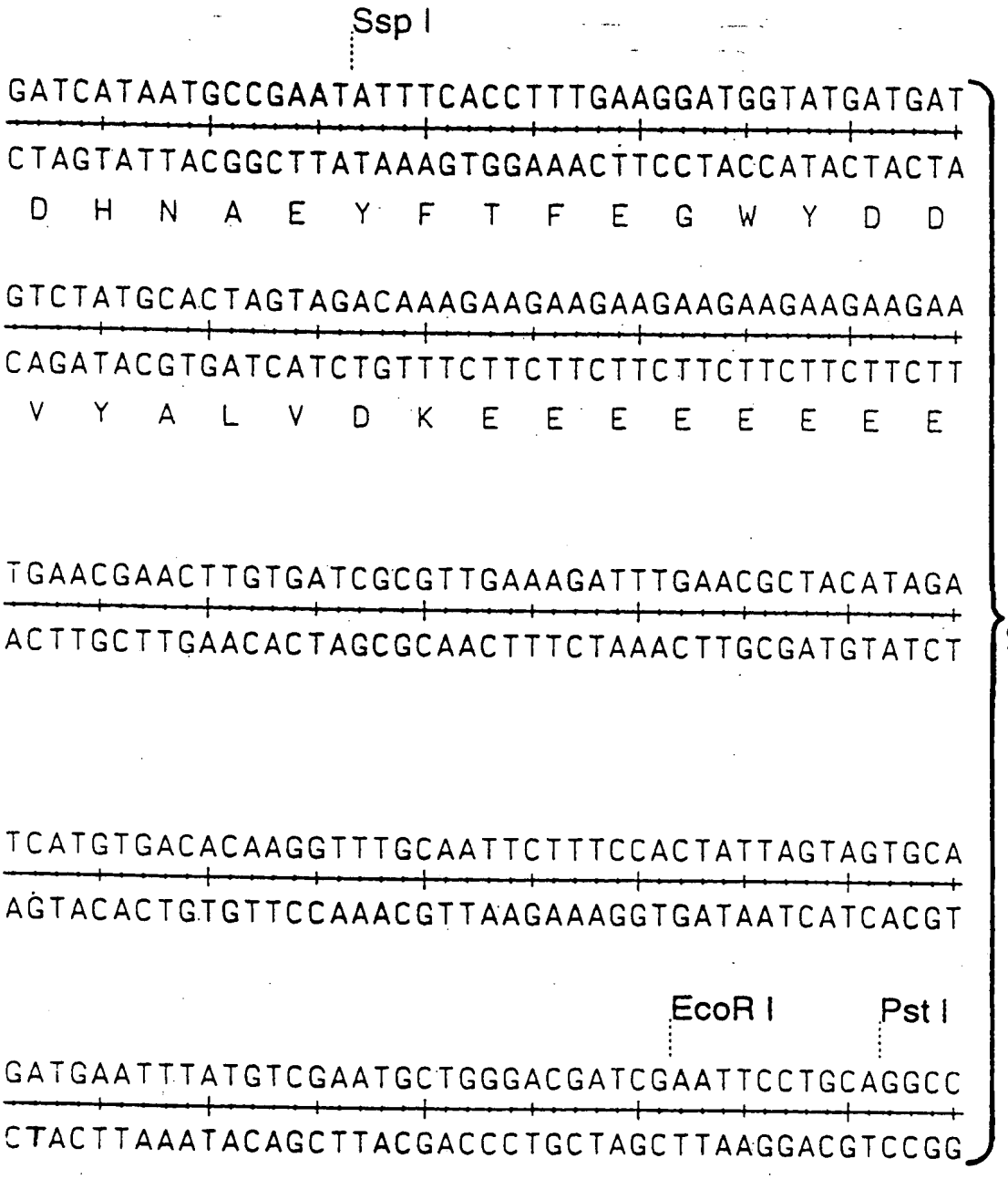

Fig 5 Sheet 12

Fig. 5 SHEET 11

```
 180       190       200       210       220
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: ::DP L. Y :H: .R .:Y . : I:KYEG LE.F::GY K.GF.R
LLNLDPTLEPYLDHFRHRMKRYVDQKMLIEKYEGPLEEFAQGYLKFGFNR
  100       110       120       130       140
 230       240       250       260       270
SATGITYREWALGAQSAALIGDFNNWDANADIMTRNEFGVWEIFLPNNVD
... I.YREWA :AQ.A.:IGDFN.W:::.::M.:::FGVW.I :P: VD
EDGCIVYREWAPAAQEAEVIGDFNGWNGSNHMMEKDQFGVWSIRIPD-VD
  150       160       170       180       190
 280       290       300       310       320
GSPAIPHGSRVKIRMDTPSGV-KDSIPAWINYSLQLPDEI--PYNGIHYD
:.P. IPH. SRVK:R.. :GV D. IPAWI:Y: . :.: PY:G:.D
SKPVIPHNSRVKFRFKHGNGVWVDRIPAWIKYATADATKFAAPYDGVYWD
    200       210       220       230       240
    330       340       350       360       370
PPEEERYIFQHPRPKKPKSLRIYESHIGMSSPEPKINSYVNFRDEVLPRI
PP .ERY F:.PRP KP:: RIYE:H:GMSS:EP::NSY :F D:VLPRI
PPPSERYHFKYPRPPKPRAPRIYEAHVGMSSSEPRVNSYREFADDVLPRI
    250       260       270       280       290
    380       390       400       410       420
KKLGYNALQIMAIQEHSYYASFGYHVTNFFAPSSRFGTPDDLKSLIDKAH
K .YN::Q:MAI EHSYY:SFGYHVTNFFA S:R:G.P:DLK LIDKAH
KANNYNTVQLMAIMEHSYYGSFGYHVTNFFAVSNRYGNPEDLKYLIDKAH
    300       310       320       330       340
    430       440       450       460       470
ELGIVVLMDIVHSHASNNTLDGLNMFDC---TDSCYFHSGARGYHWMWDS
.LG: VL:D:VHSHASNN. DGLN FD :::.YFH:G.RGYH :WDS
SLGLQVLVDVVHSHASNNVTDGLNGFDIGQGSQESYFHAGERGYHKLWDS
    350       360       370       380       390
        480       490       500       510       520
RLFNYGNWEVLRYLLSNARWWLDAFKFDGFRFDGVTSMMYIHHGLSVGFT
RLFNY:NWEVLR:LLSN RWWL:.::FDGFRFDG:TSM:Y:HHG:::GFT
RLFNYANWEVLRFLLSNLRWWLEEYNFDGFRFDGITSMLYVHHGINMGFT
    400       410       420       430       440
        530       540       550       560       570
GNYEEYFGLATDVDAVVYLMLVNDLIHGLFPDAITIGEDVSGMPTFCIPV
GNY:EYF: ATDVDAVVYLML.N:LIH :FPDA..I:EDVSGMP.:. PV
GNYNEYFSEATDVDAVVYLMLANNLIHKIFPDATVIAEDVSGMPGLSRPV
    450       460       470       480       490
        580       590       600       610       620
QEGGVGFDYRLHMAIADKRIELLK-KRDEDWRVGDIVHTLTNRRWSEKCV
 EGG:GFDYRL MAI:DK:I: LK K.DEDW.: ::. :LTNRR.:EKC:
SEGGIGFDYRLAMAIPDKWIDYLKNKNDEDWSMKEVTSSLTNRRYTEKCI
    500       510       520       530       540
```

Fig. 6 SHEET 1

SUBSTITUTE SHEET (RULE 26)

```
          ↓10         ↓20         ↓30         ↓40
MVYTLSGVRFPTVPSVYKSNGFSSNGDRRNANVSVFLKKH--SLSRKILA
MVYT:SG:RFP.:PS:.KS   :  .  DRR.::  S FLK::   S:SR.  L
MVYTISGIRFPVLPSLHKS---TLRCDRRASSHSFFLKNNSSSFSRTSLY
          ↑10         ↑20         ↑30         ↑40
 ↓50         ↓60         ↓70         ↓80         ↓90
EKSSYNSEFRPSTVAASGKVLVPGTQSDSSSSSTDQFEFTETSPENSPAS
.K S :SE ::ST:A. S:KVL:P..Q D:S S :DQ:E .:.::E::..
AKFSRDSETKSSTIAESDKVLIPEDQ-DNSVSLADQLENPDITSEDAQNL
 ↑50         ↑60         ↑70         ↑80         ↑90
 ↓100        ↓110        ↓120        ↓130        ↓140
TDVDSSTMEHASQIKTENDDVEPSSDLTGSVEELDFASSLQLQEGGKLEE
.D:    TM.::::  :.:  :....:  .::.:.  ..:.  :S :::::.:.:  .
EDL---TMKDGNKYNID-ESTSSYREVGDEKGSVTSSSLVDVNTDTQ--A
       ↑100        ↑110        ↑120        ↑130        ↑140
 ↓150        ↓160        ↓170        ↓180        ↓190
SKTLNTSEETIIDESDRIRERGIPPPGLGQKIYEIDPLLTNYRQHLDYRY
.KT   S:..:   :.  :I    IPPPG GQKIYEIDPLL . .RQHLD:RY
KKTSVHSDKKVKVDKPKI----IPPPGSGQKIYEIDPLLQAHRQHLDFRY
          ↑150        ↑160        ↑170        ↑180
 ↓200        ↓210        ↓220        ↓230        ↓240
SQYKKLREAIDKYEGGLEAFSRGYEKMGFTRSATGITYREWALGAQSAAL
:QYK::RE. IDKYEGGL:AFSRGYEK.GFTRSATGITYREW:  GA:SAAL
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:GDFNNW::NAD:MT:.FGVWEIFLPNN.DGSP:IPHGSRVKI:MDTPS
VGDFNNWNPNADVMTKDAFGVWEIFLPNNADGSPPIPHGSRVKIHMDTPS
   ↑240        ↑250        ↑260        ↑270        ↑280
 ↓300        ↓310        ↓320        ↓330        ↓340
GVKDSIPAWINYSLQLPDEIPYNGIHYDPPEEERYIFQHPRPKKPKSLRI
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GIKDSIPAWIKFSVQAPGEIPYNGIYYDPPEEEKYVFKHPQPKRPQSIRI
   ↑290        ↑300        ↑310        ↑320        ↑330
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YESHIGMSSPEPKINSYVNFRDEVLPRIKKLGYNALQIMAIQEHSYYASF
YESHIGMSSPEPKIN:Y.NFRD:VLPRIKKLGYNA:QIMAIQEHSYYASF
YESHIGMSSPEPKINTYANFRDDVLPRIKKLGYNAVQIMAIQEHSYYASF
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 ↓400        ↓410        ↓420        ↓430        ↓440
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Fig. 7 SHEET 1

SUBSTITUTE SHEET (RULE 26)

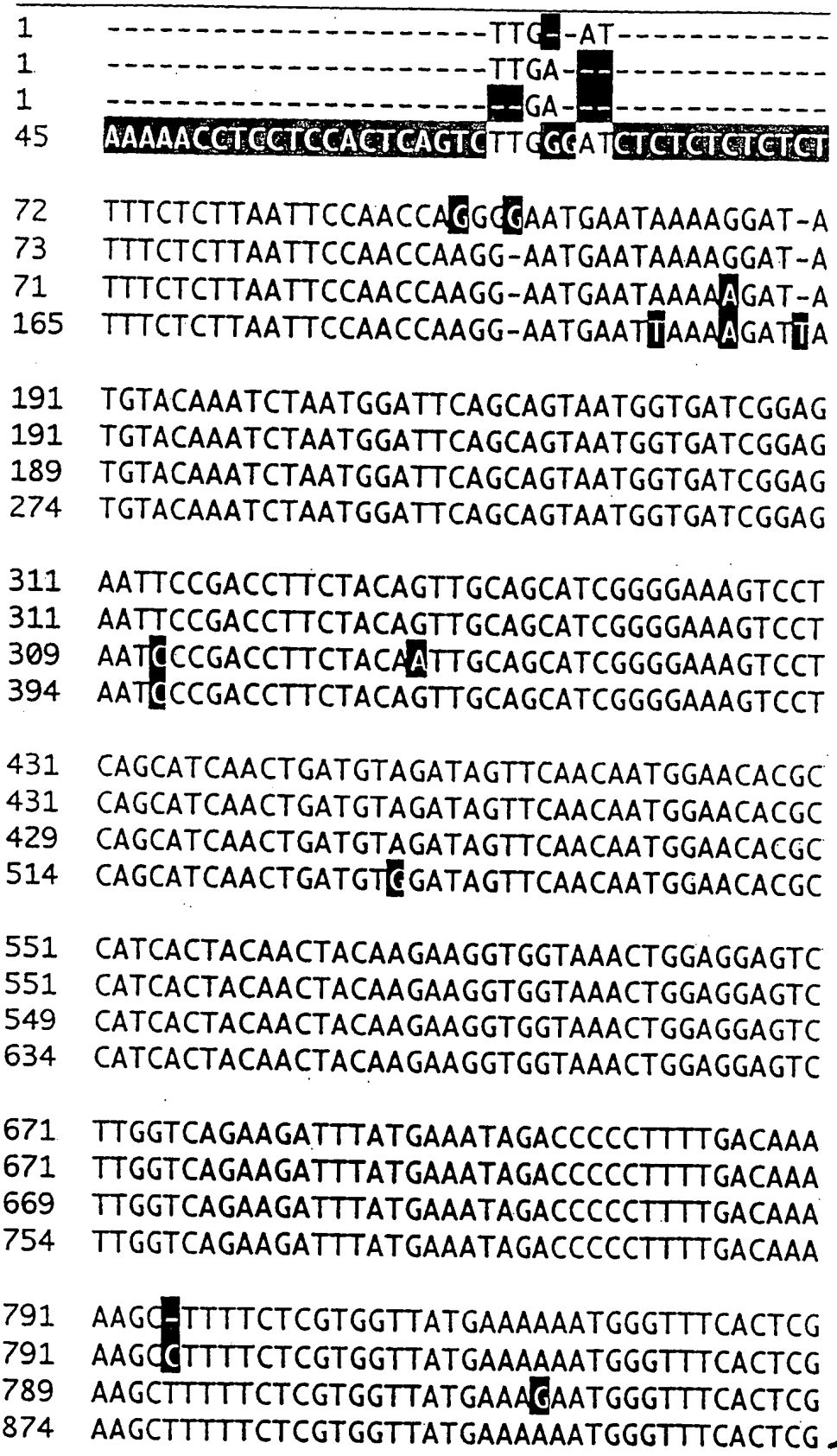

Fig.8 Sheet 2

Fig. 8 SHEET 1

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Fig. 8
SHEET 3

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Fig. 8
Sheet 6

Fig. 8
SHEET 5

```
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1870 GGGGGTGTTGGCTTTGACTATCGGCTGCATATGGCAATTGC
1869 GGGGGTGTTGGCTTTGACTATCGGCTGCATATGGCAATTGC
1953 GGGGGTGTTGGCTTTGACTATCGGCTGCATATGGCAATTGC

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2108 CCGCCAACATCATTAATAGATCGTGGGATAGCATTGCACAA
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2109 CCGTCAACATCATTAATAGATCGTGGGATAGCATTACACAA
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Fig.8
Sheet 8

Fig.8
SHEET 7

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Fig. 8
SHEET 9

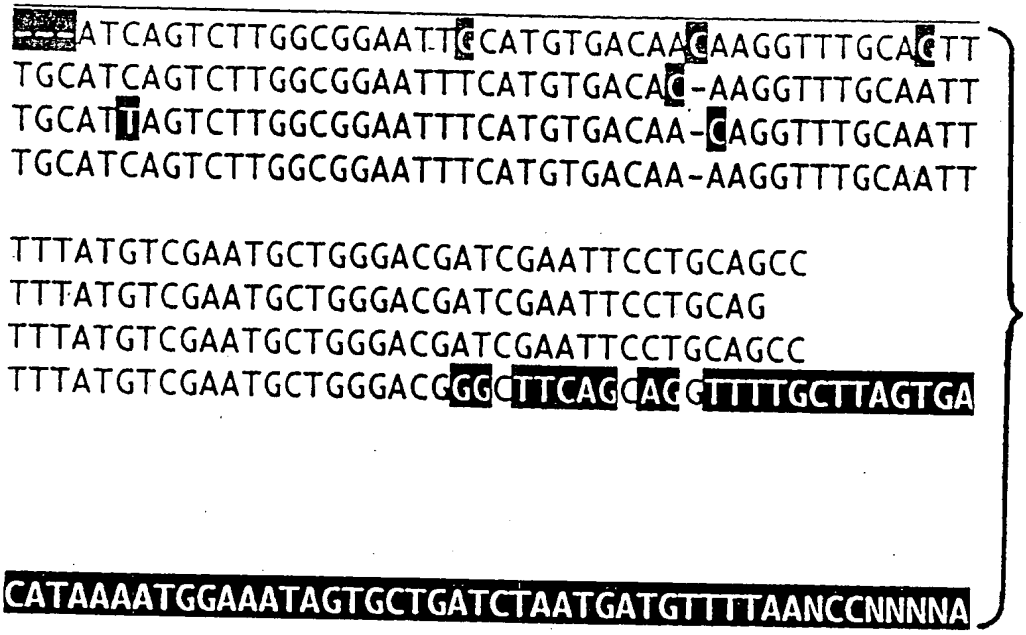

Fig. 8 Sheet 12

Fig. 8 SHEET 11

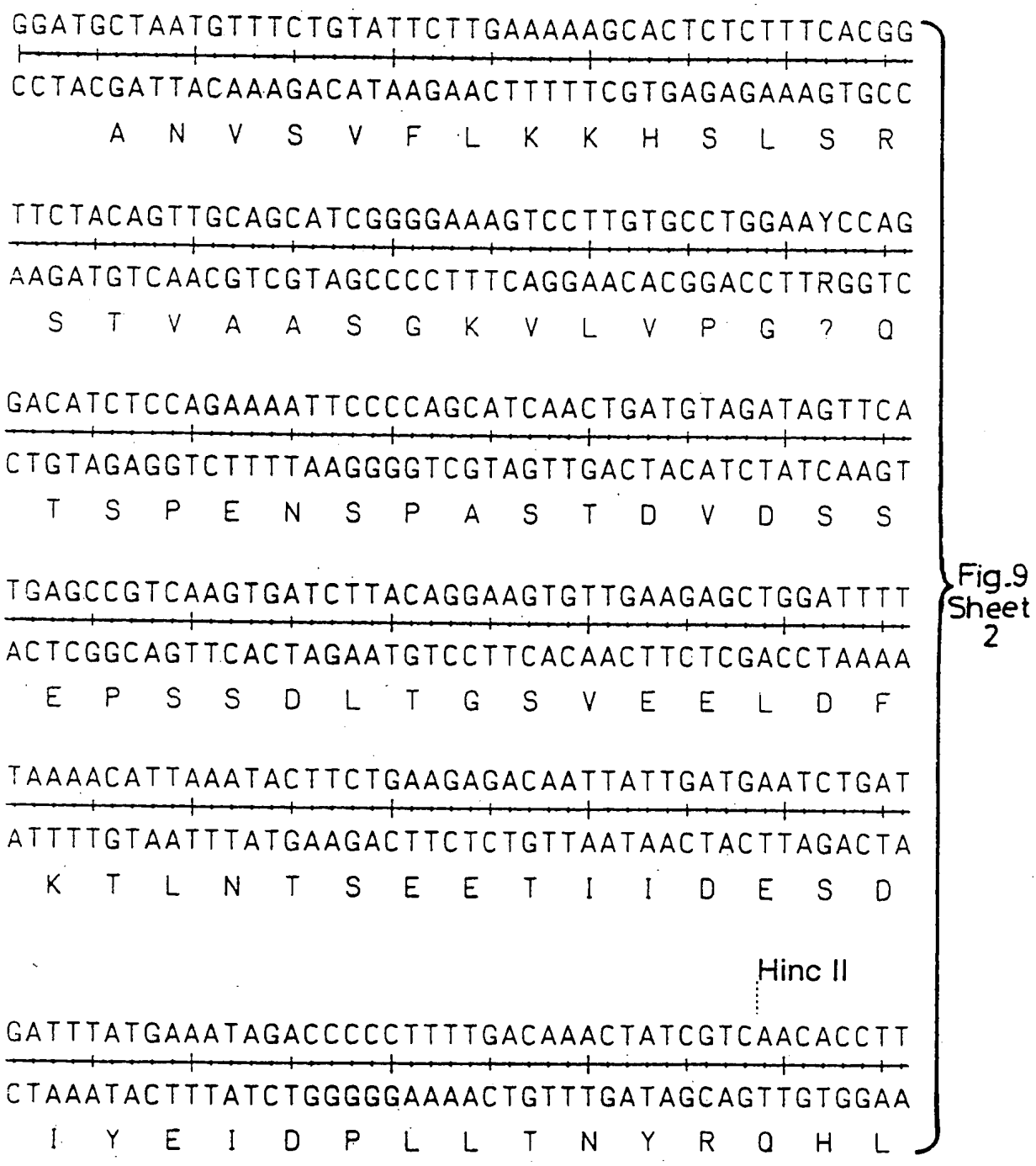

Fig.9 Sheet 2

Fig. 9 SHEET 1

HinD III

```
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GTTCATACTCCCACCAAACCTTCGAAAAAGAGCACCAATACTTTTT
  K  Y  E  G  G  L  E  A  F  S  R  G  Y  E  K
```

Pvu II

```
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CCGAGGACCACGGGTCAGTCGACGGGAGTAACCTCTAAAGTTGTTA
  A  P  G  A  Q  S  A  A  L  I  G  D  F  N  N

CTGGGAGATTTTTCTGCCAAATAATGTGGATGGTTCTCCTGCAATT
GACCCTCTAAAAAGACGGTTTATTACACCTACCAAGAGGACGTTAA
  W  E  I  F  L  P  N  N  V  D  G  S  P  A  I

TGTTAAGGATTCCATTCCTGCTTGGATCAACTACTCTTTACAGCTT
ACAATTCCTAAGGTAAGGACGAACCTAGTTGATGAGAAATGTCGAA
  V  K  D  S  I  P  A  W  I  N  Y  S  L  Q  L

AGAGGAGAGGTATRTCTTCCAACACCCACGGCCAAAGAAACCAAAG
TCTCCTCTCCATAYAGAAGGTTGTGGGTGCCGGTTTCTTTGGTTTC
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Fig.9 Sheet 4

Fig.9 SHEET 3

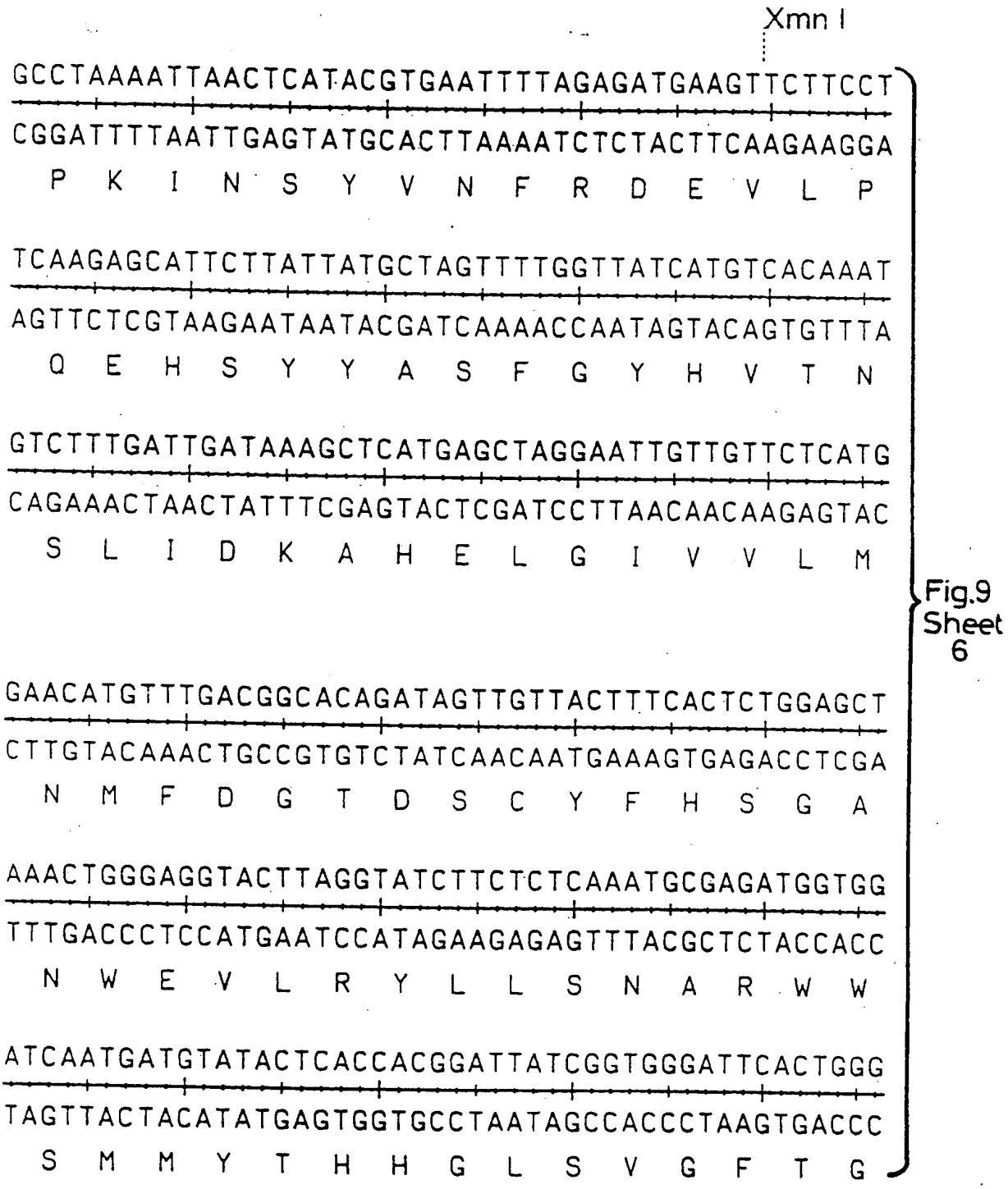

Fig.9 Sheet 6

Fig. 9 SHEET 5

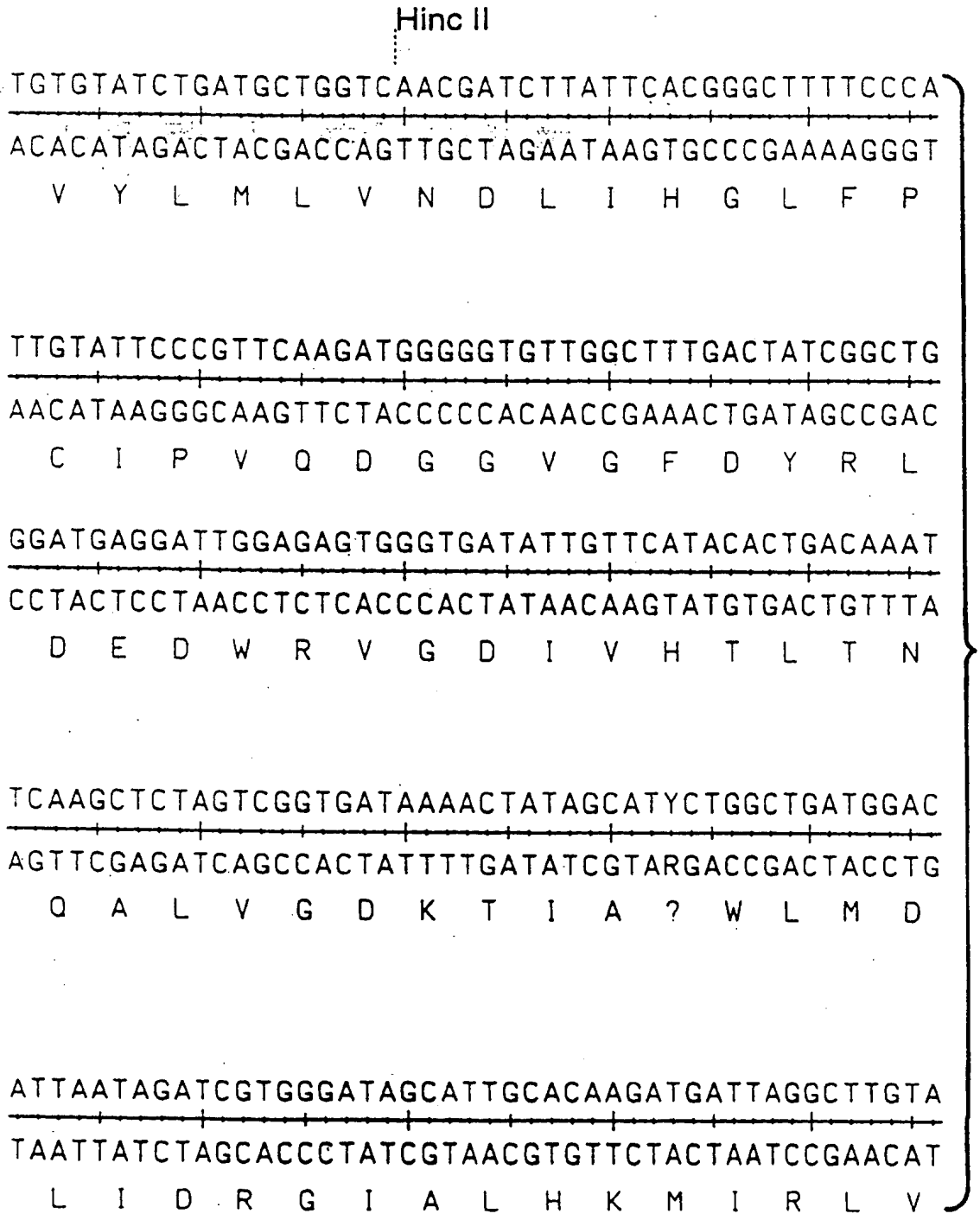

Fig.9 Sheet 8

Fig. 9 SHEET 7

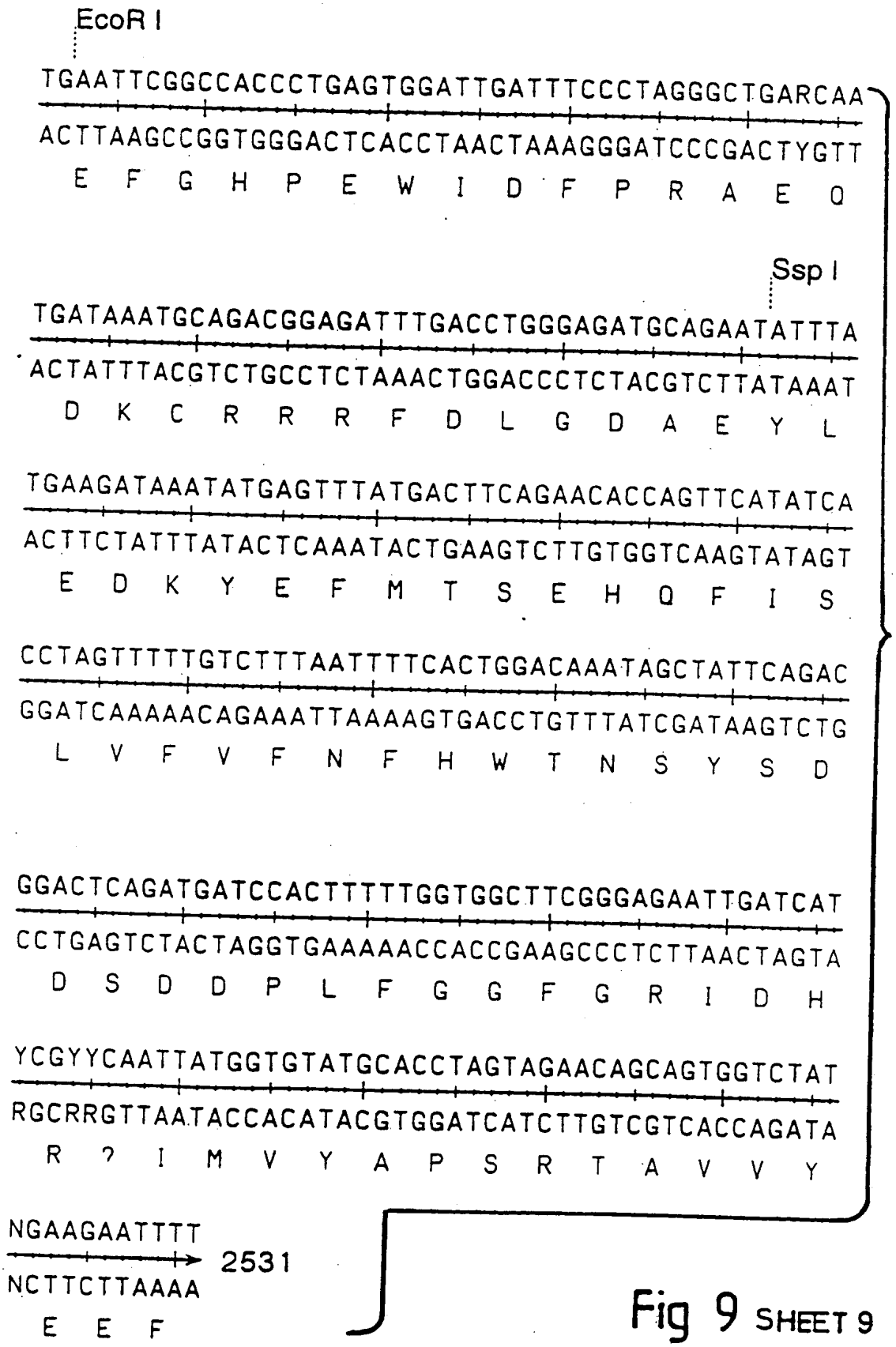

Fig 9 Sheet 10

Fig 9 SHEET 9

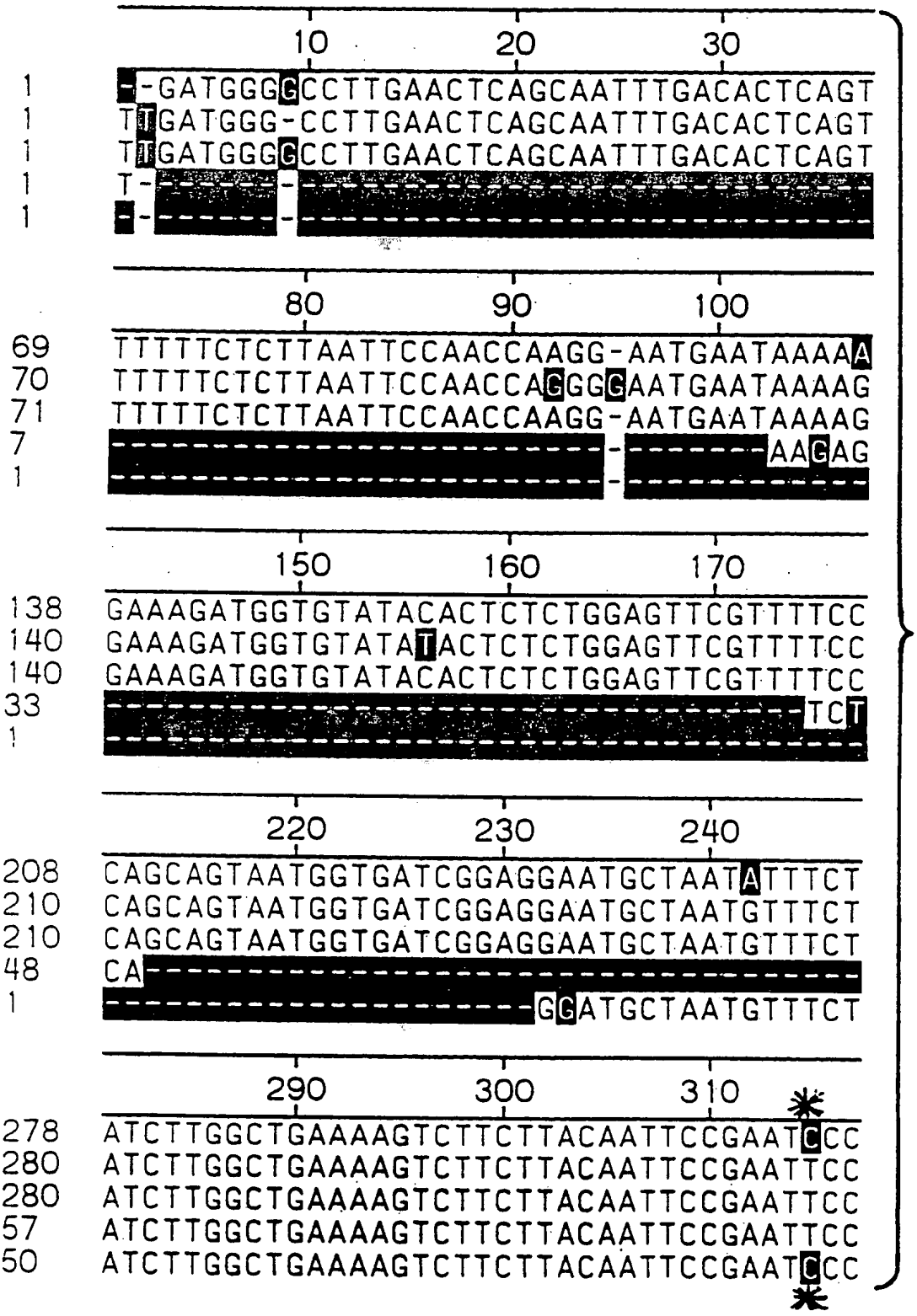

Fig.10 Sheet 2

Fig. 10 SHEET 1

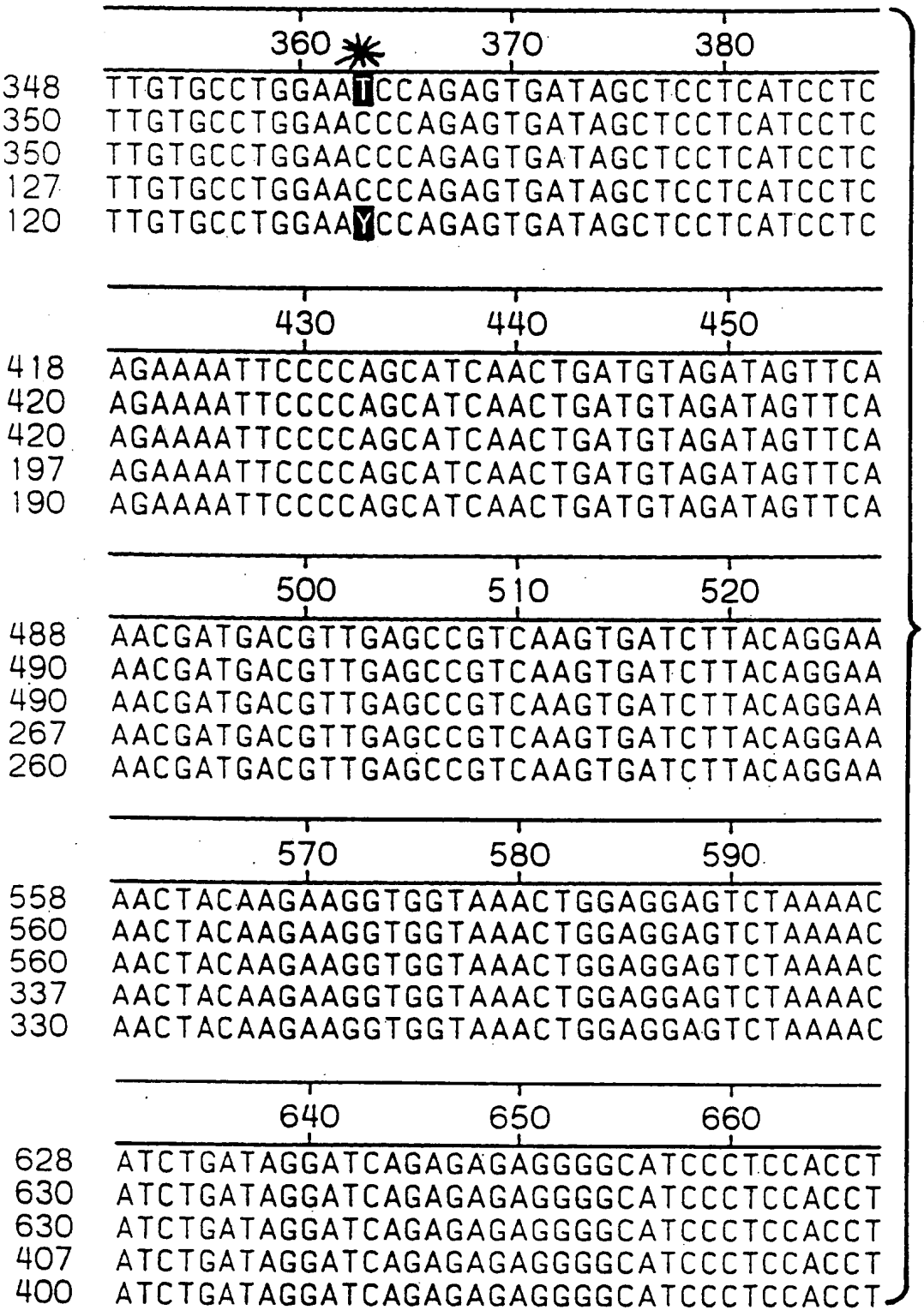

Fig. 10 Sheet 4

Fig. 10 SHEET 3

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700  CTTTTGACAAACTATCGTCAACACCTTGATTACAGGT
477  CTTTTGACAAACTATCGTCAACACCTTGATTACAGGT
470  CTTTTGACAAACTATCGTCAACACCTTGATTACAGGT

                780        790        800
768  ACAAGTATGAGGGTGGTTTGGAAGCTTTTTCTCGTGG
770  ACAAGTATGAGGGTGGTTTGGAAGC-TTTTCTCGTGG
770  ACAAGTATGAGGGTGGTTTGGAAGCCTTTTCTCGTGG
547  ACAAGTATGAGGGTGGTTTGGAAGCTTTTTCTCGTGG
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                850        860        870
838  AGGTATCACTTACCGTGAGTGGGCTCCTGGTGCCCAG
839  AGGTATCACTTACCGTGAGTGGGCTCCTGGTGCCCAG
840  AGGTATCACTTACCGTGAGTGGGCTCTTGGTGCCCAG
617  AGGTATCACTTACCGTGAGTGGGCTCCTGGTGCCCAG
610  AGGTATCACTTACCGTGAGTGGGCTCCTGGTGCCCAG

                920        930        940
908  GACGCAAATGCTGACTTTATGACTCGGAATGAATTTG
909  GACGCAAATGCTGACATTATGACTCGGAATGAATTTG
910  GACGCAAATGCTGACATTATGACTCGGAATGAATTTG
687  GACGCAAATGCTGACATTATGACTCGGAATGAATTTG
680  GACGCAAATGCTGACATTATGACTCGGAATGAATTTG

                990        1000       1010
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Fig.10 Sheet 6

Fig. 10 SHEET 5

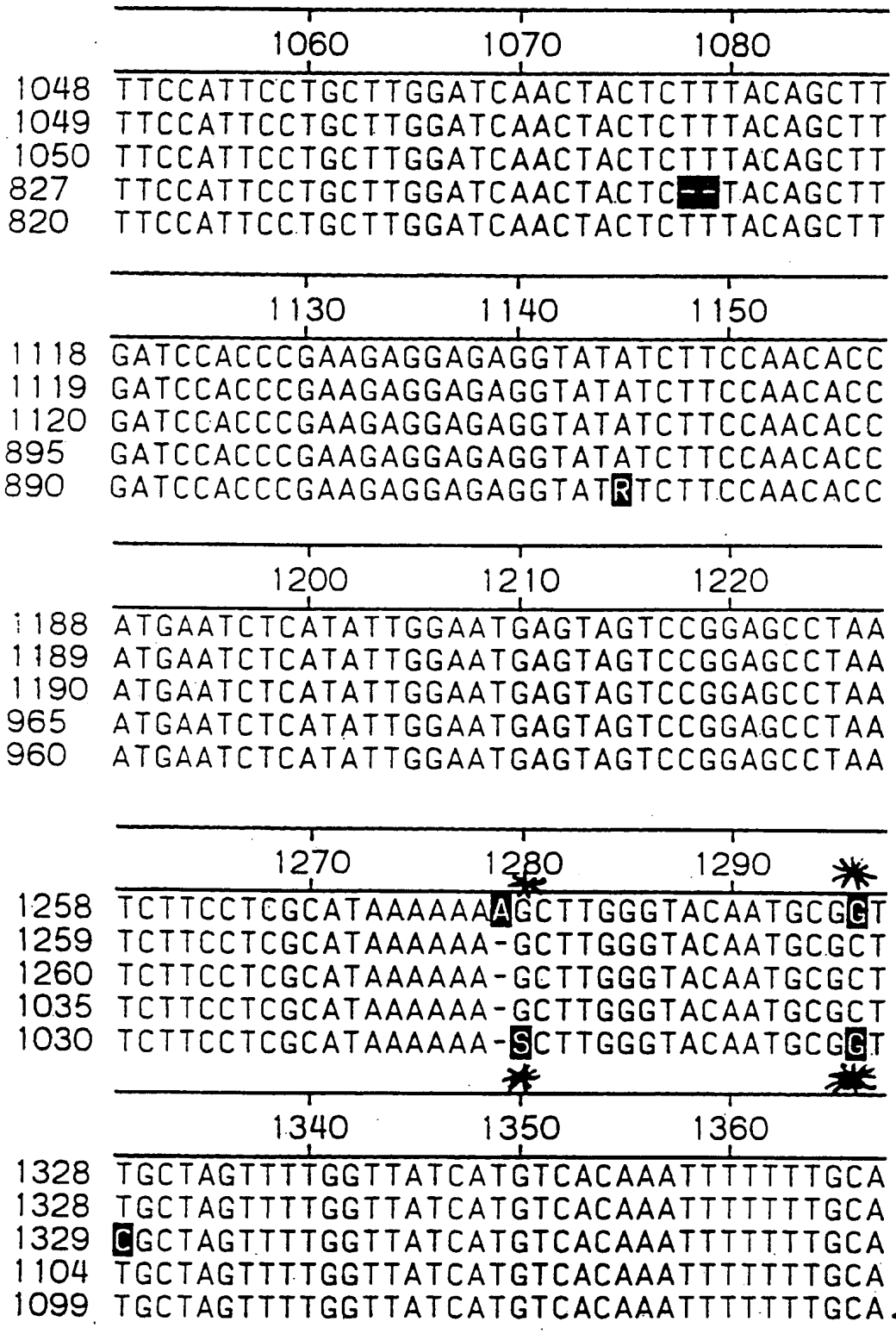

Fig.10 Sheet 8

Fig. 10 SHEET 7

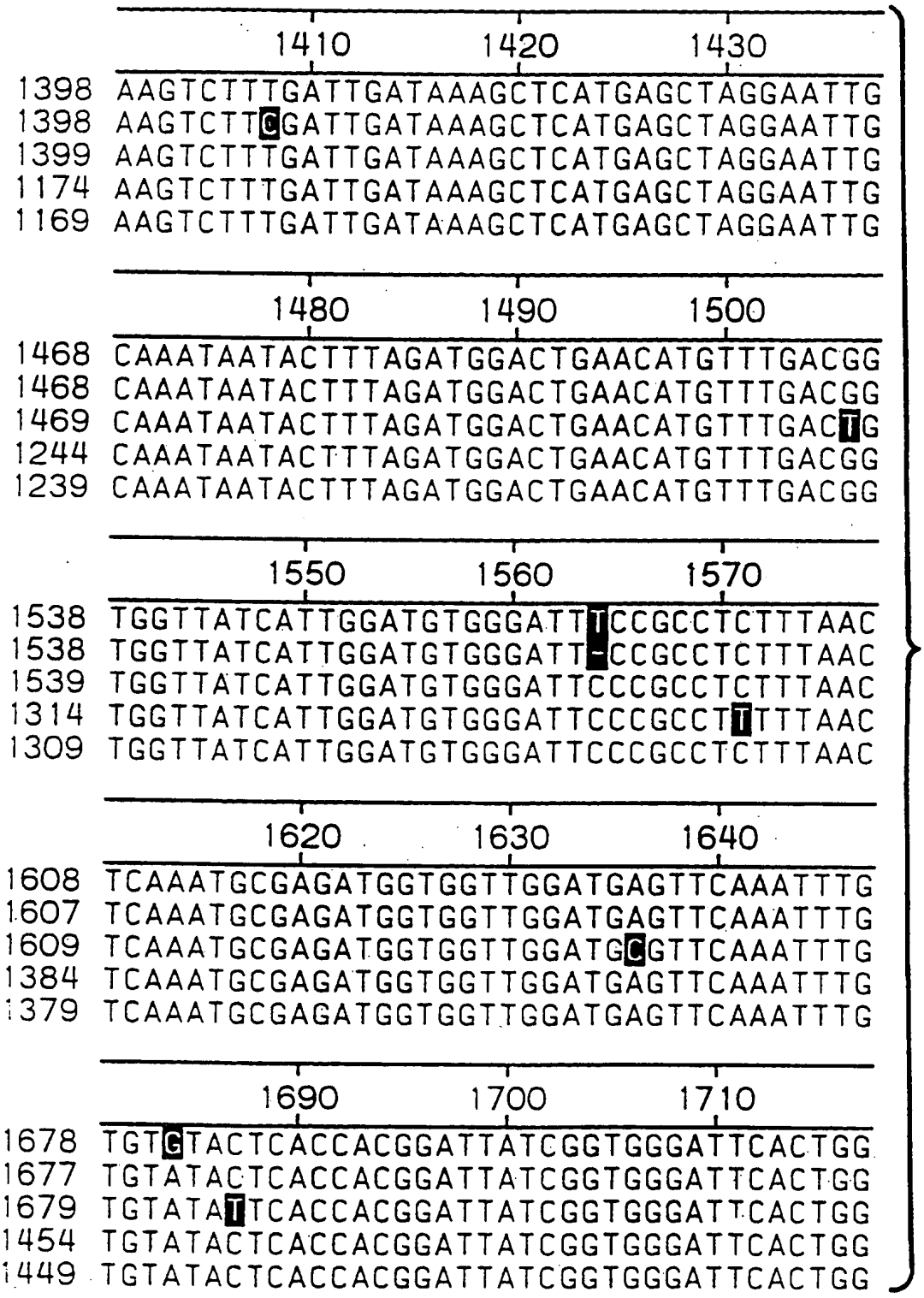

Fig. 10 Sheet 10

Fig. 10 SHEET 9

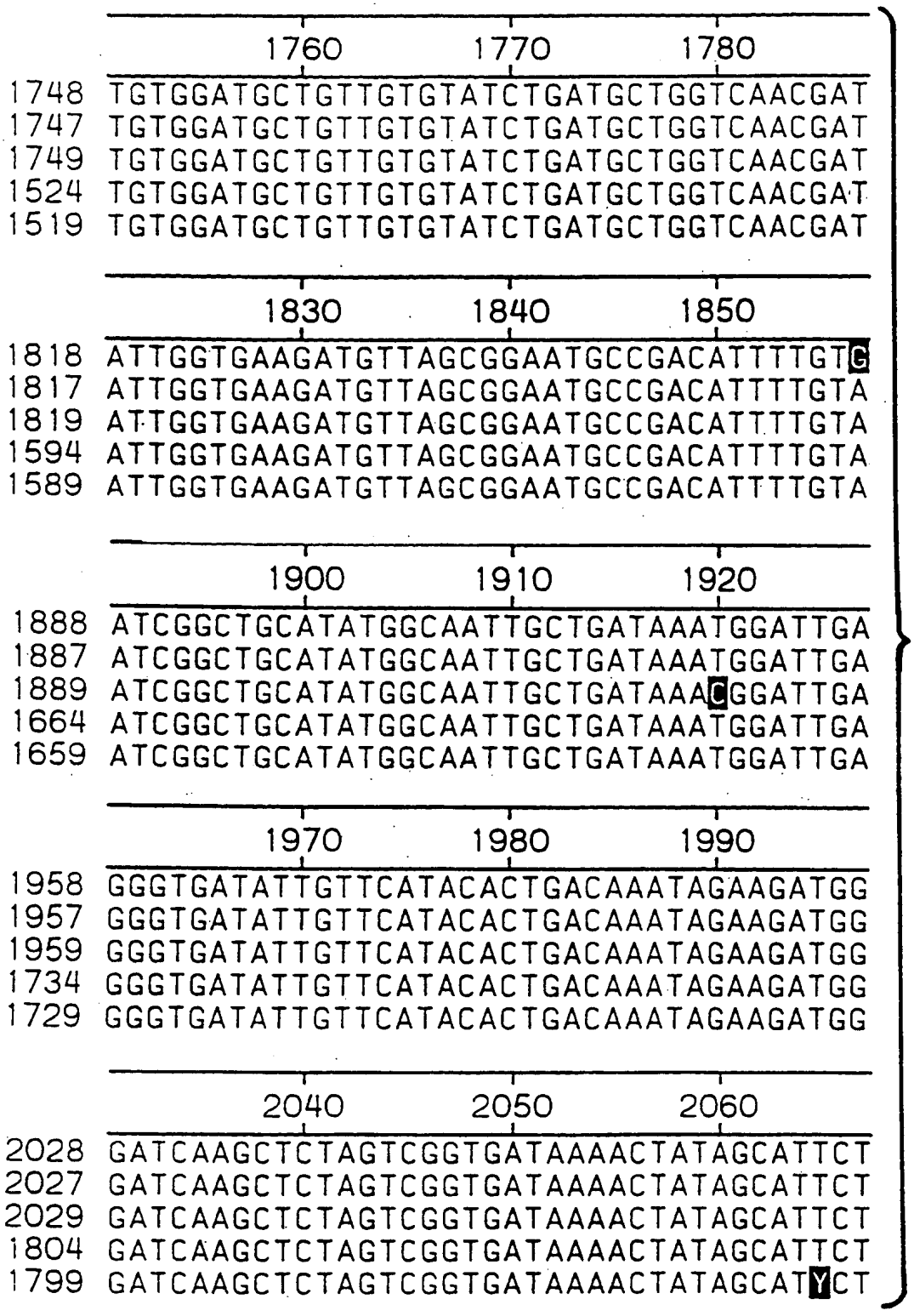

Fig.10 Sheet 12

Fig. 10 SHEET 11

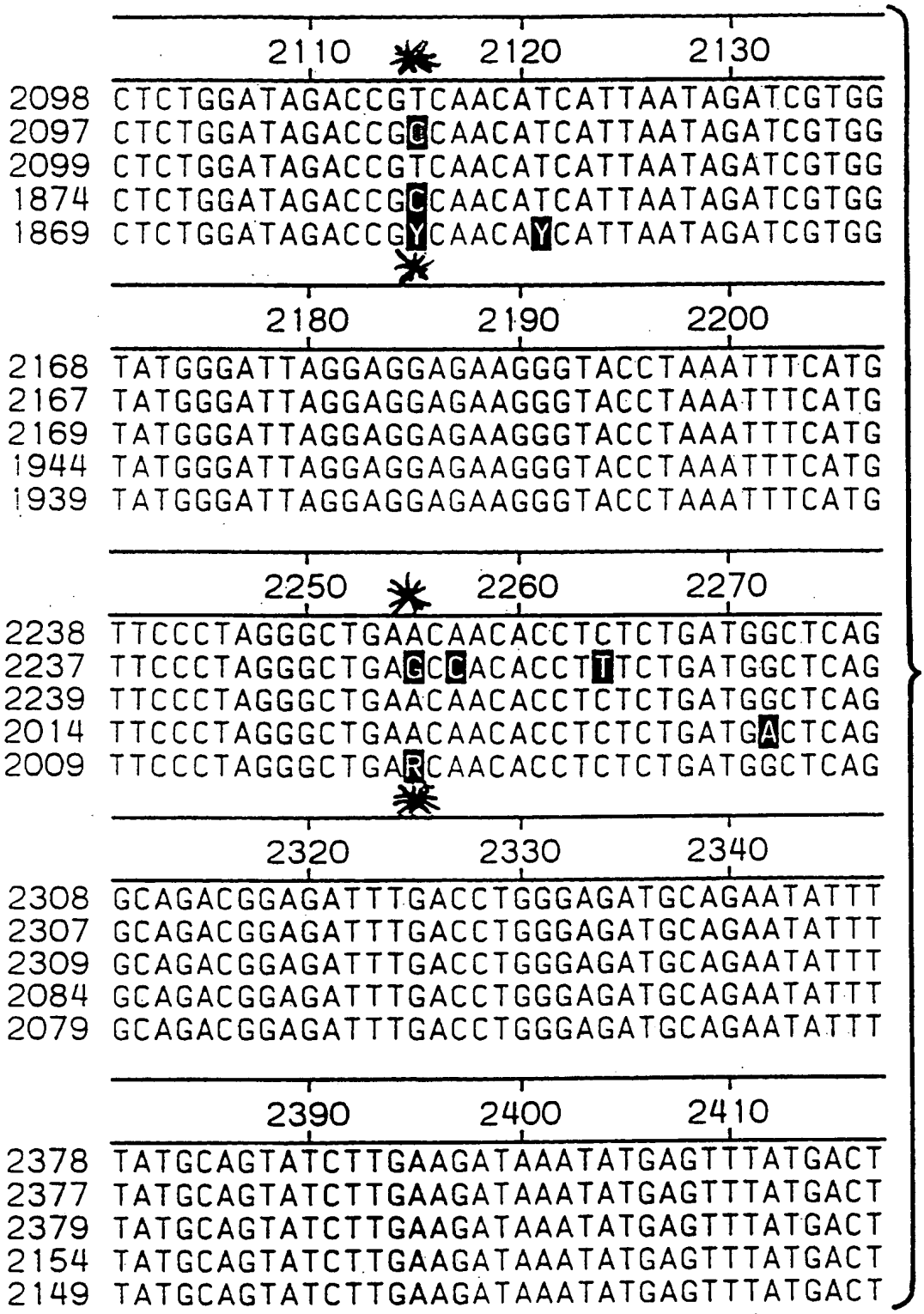

Fig.10 Sheet 14

Fig. 10 SHEET 13

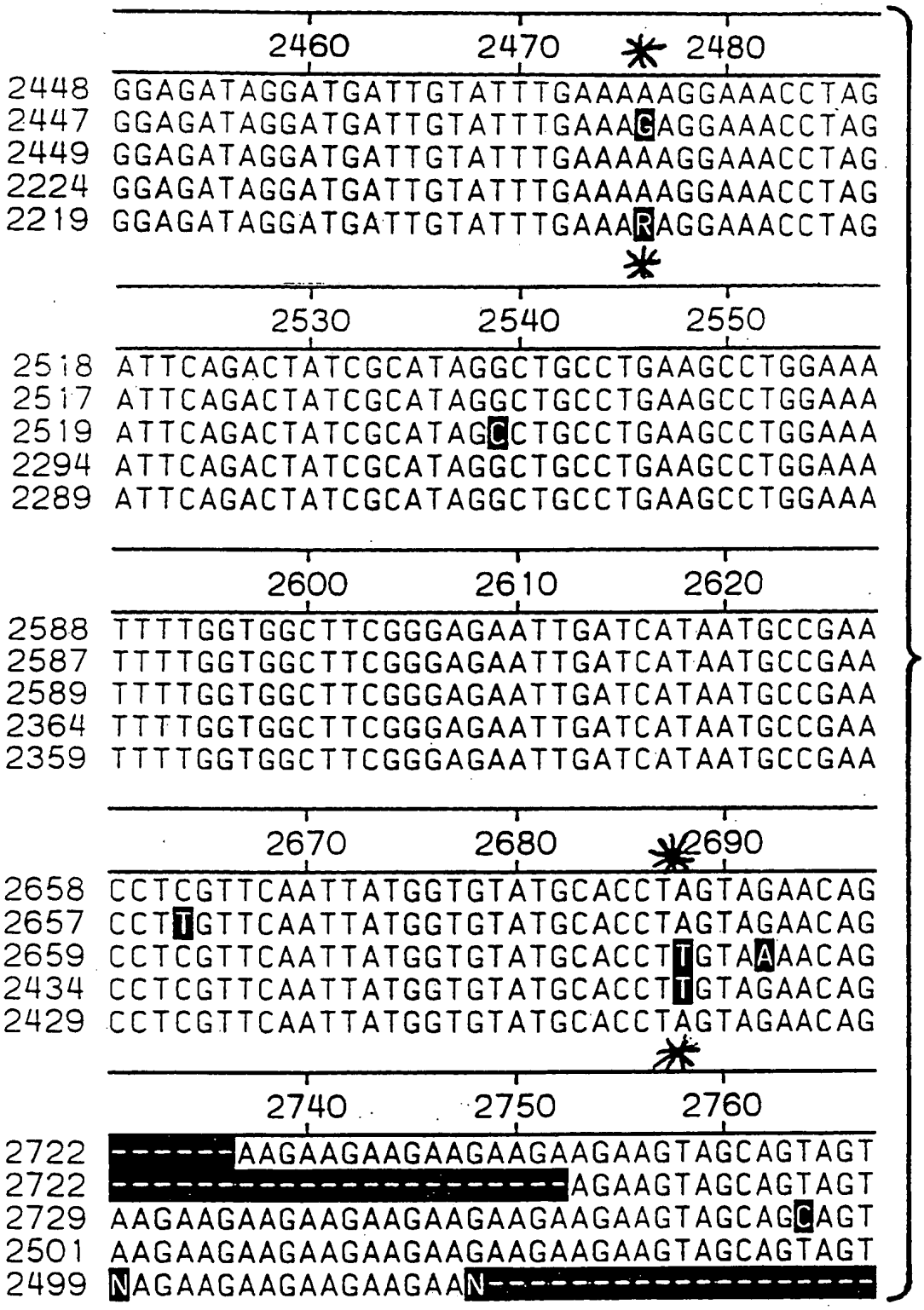

Fig. 10 Sheet 16

Fig. 10 SHEET 15

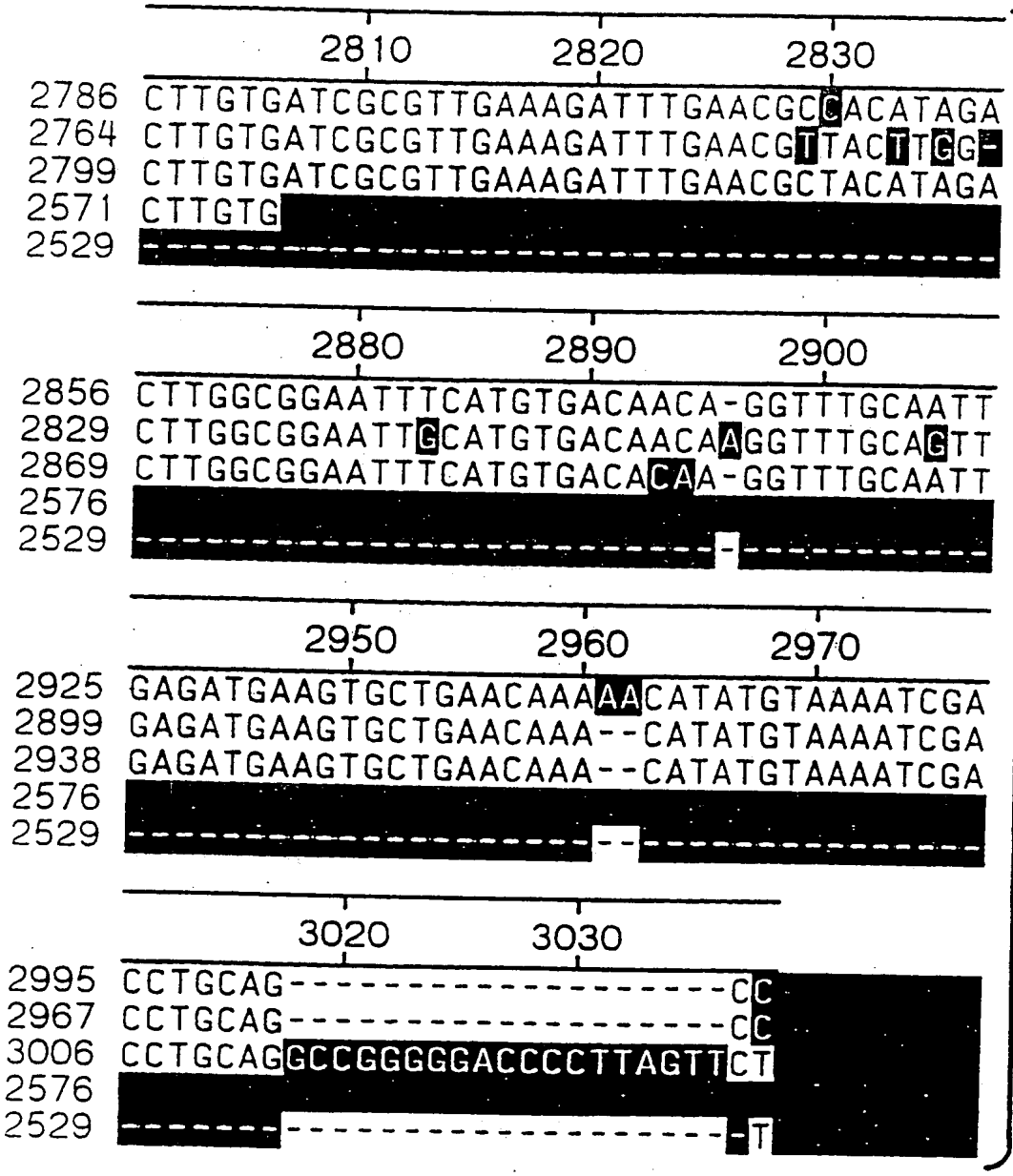

Fig. 10 Sheet 18

Fig. 10 SHEET 17

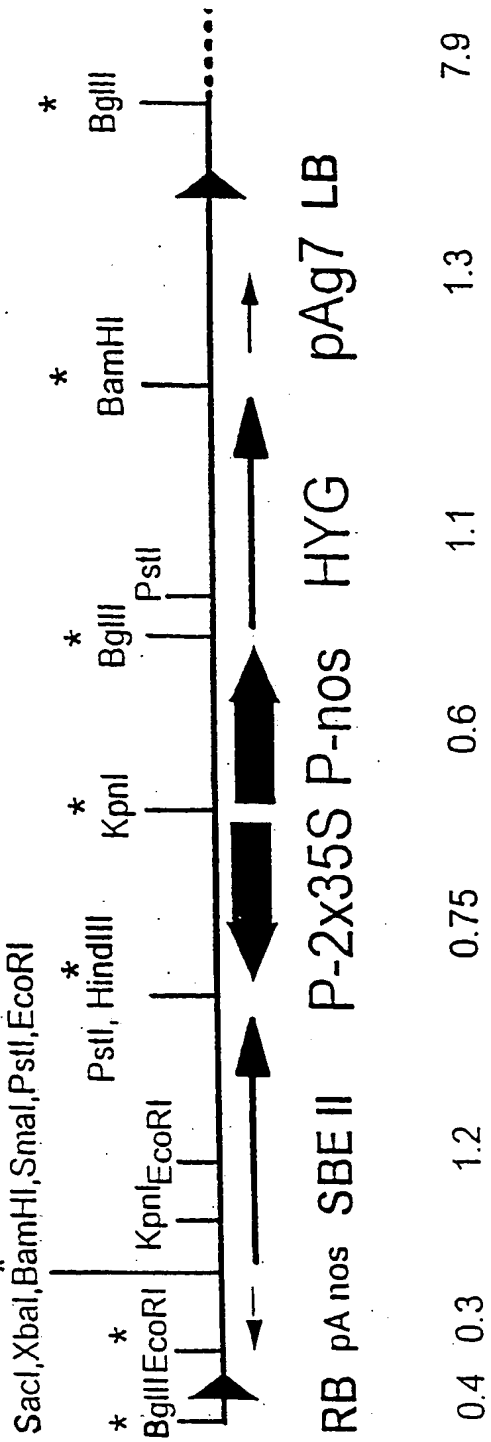

Fig. 11

Fig.12
SHEET 2

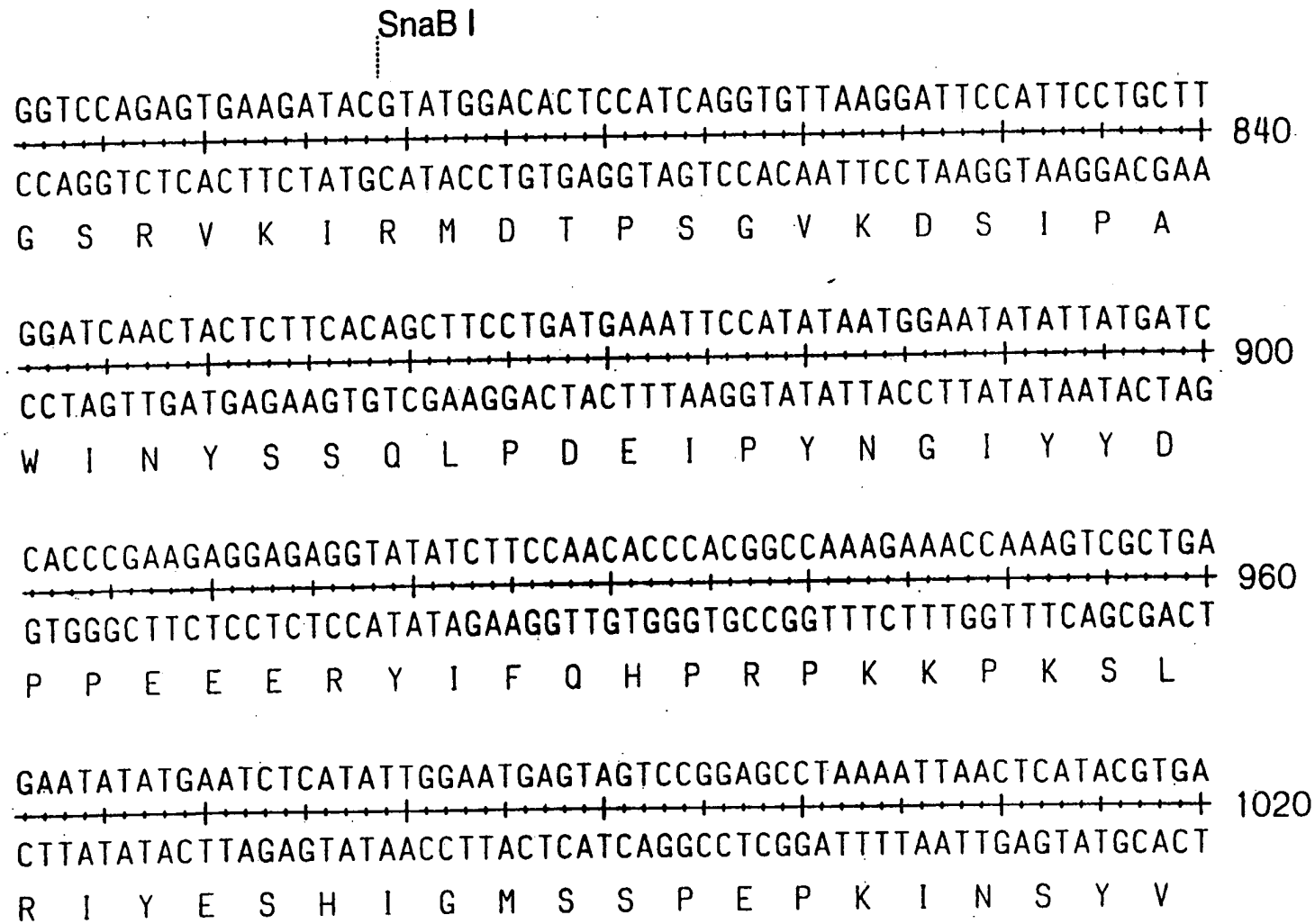

Fig. 12
SHEET 4

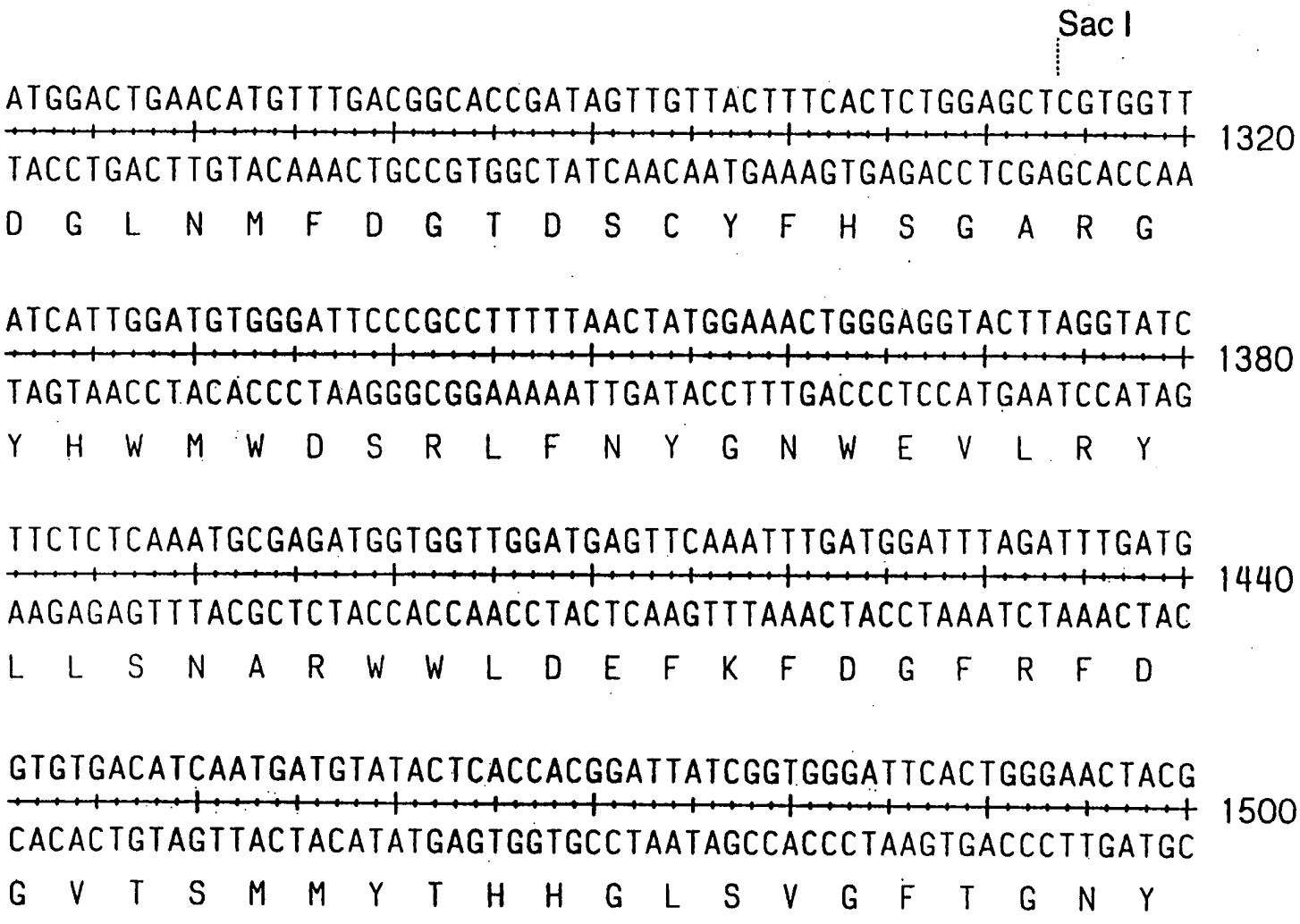

Fig. 12
SHEET 6

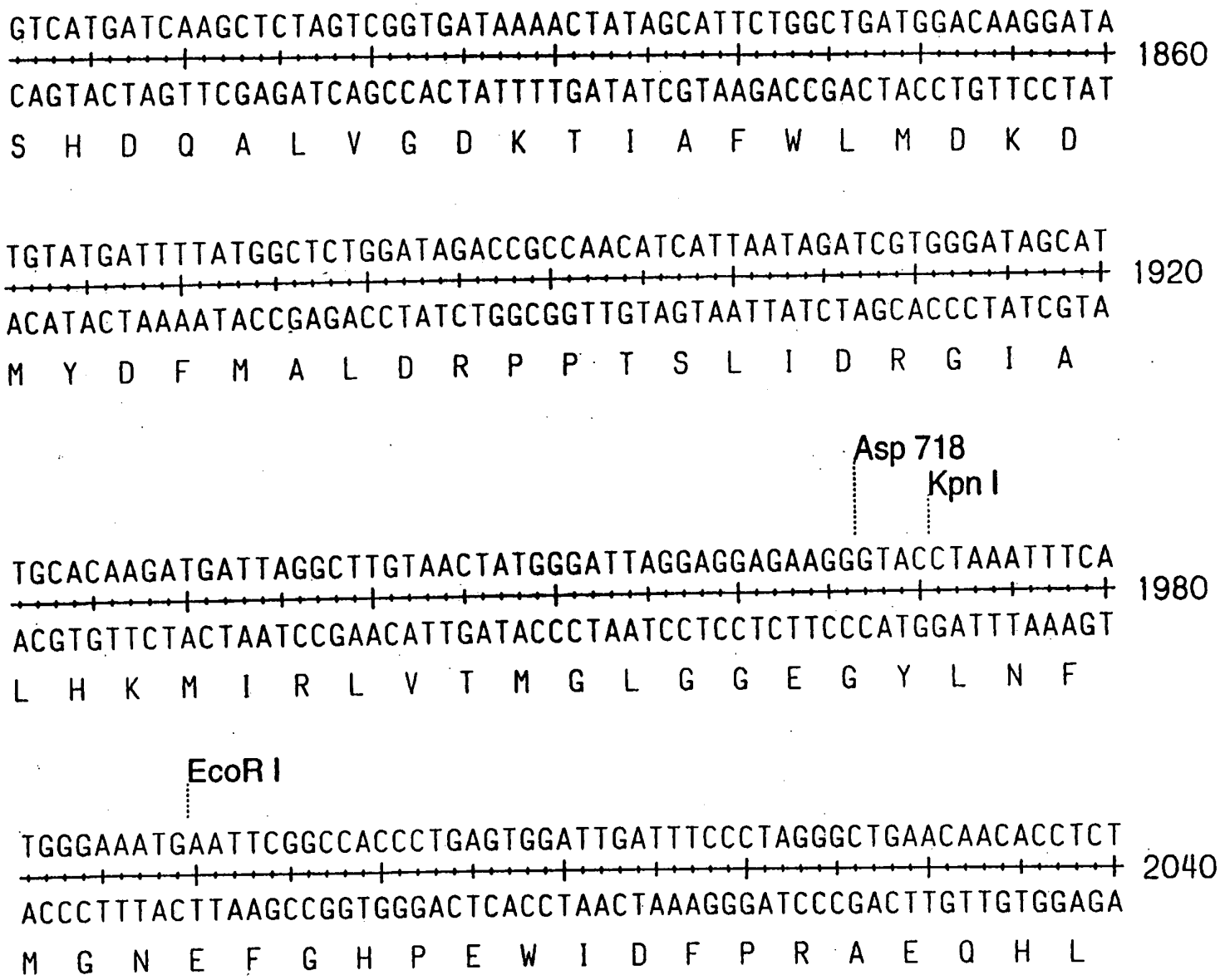

Fig 12
SHEET 8

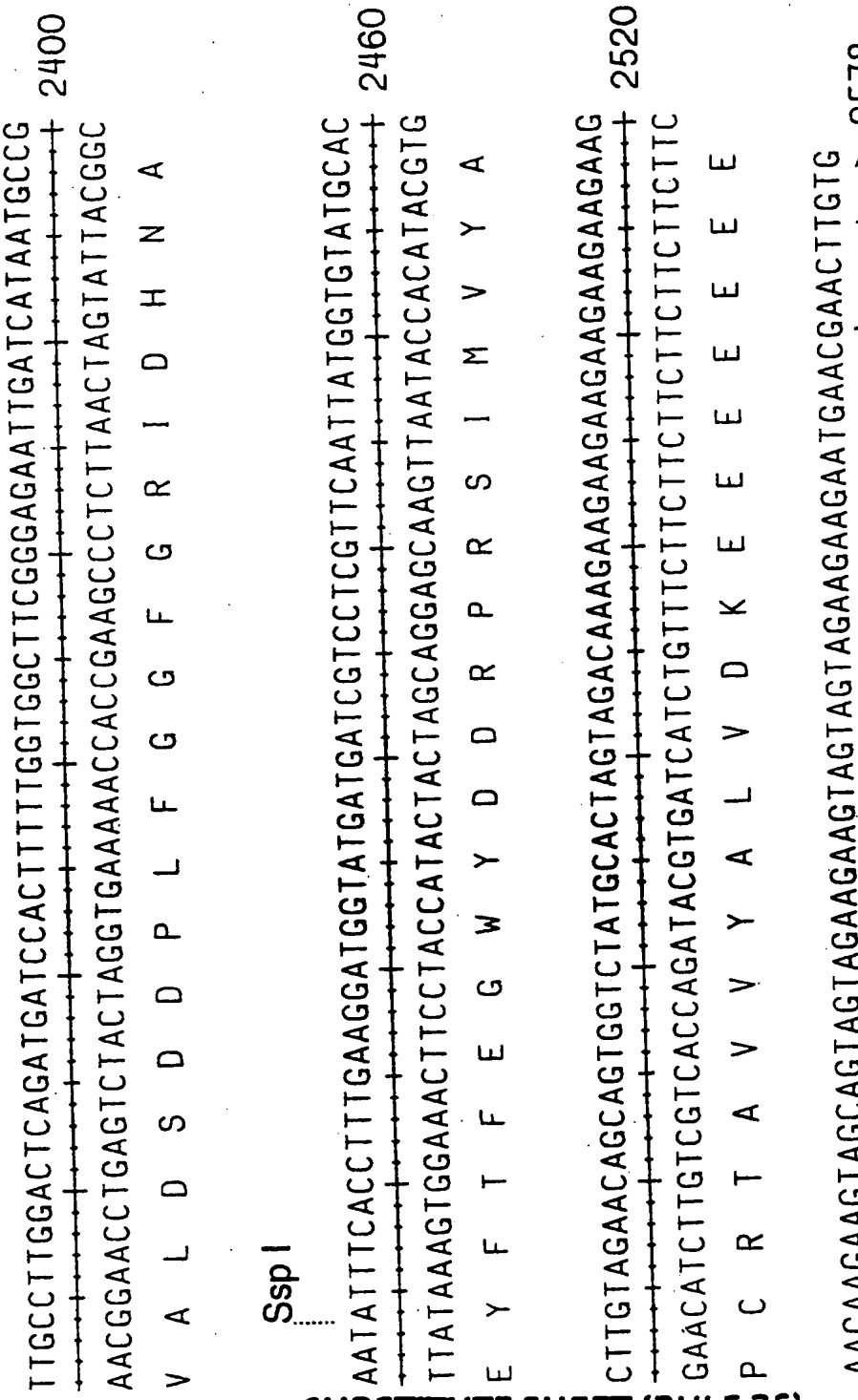

Fig 12
SHEET 10

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/82, 15/54, C08B 30/04, A01H 5/00 — A3

(11) International Publication Number: **WO 96/34968**

(43) International Publication Date: 7 November 1996 (07.11.96)

(21) International Application Number: PCT/GB96/01075

(22) International Filing Date: 3 May 1996 (03.05.96)

(30) Priority Data:

9509229.2	5 May 1995 (05.05.95)	GB
9607409.1	10 April 1996 (10.04.96)	GB

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(74) Agent: KEITH W. NASH & CO.; 90-92 Regent Street, Cambridge CB2 1DP (GB).

(81) Designated States: AU, BR, CA, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

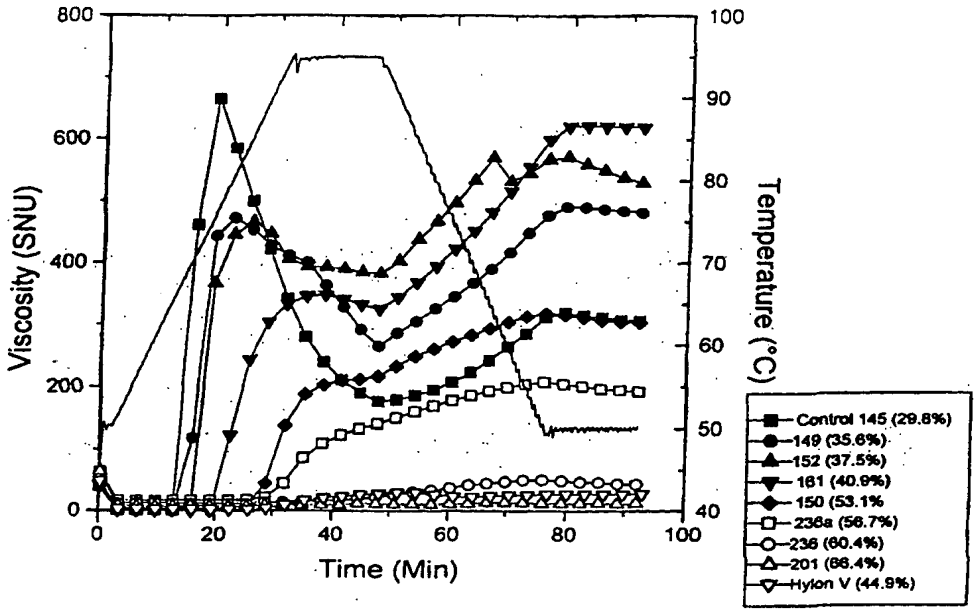
Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(88) Date of publication of the international search report: 5 December 1996 (05.12.96)

(54) Title: IMPROVEMENTS IN OR RELATING TO PLANT STARCH COMPOSITION



(57) Abstract

Disclosed is a nucleotide sequence encoding an effective portion of a class A starch branching enzyme (SBE) obtainable from potato plants, or a functional equivalent thereof, together with, inter alia, a corresponding polypeptide, a method of altering the characteristics of a plant, a plant having altered characteristics; and starch, particularly starch obtained from a potato plant, having novel properties.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 96/01075

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 C12N15/54 C08B30/04 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C08B A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THE PLANT JOURNAL, vol. 7, no. 1, January 1995, pages 3-15, XP002014042 BURTON, R.A., ET AL.: "Starch branching enzymes belonging to distinct enzyme families are differentially expressed during pea embryo development" see the whole document ---	28-32, 34,39-41
X	DATABASE WPI Section Ch, Week 9442 Derwent Publications Ltd., London, GB; Class C06, AN 94-337418 XP002014047 & JP,A,06 261 767 (MITSUI GYOSAI SHOKUBUTSU BIO KENKYUSHO) , 20 September 1994 see abstract ---	28-37, 39-42, 48-50

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Date of the actual completion of the international search	Date of mailing of the international search report
23 September 1996	23. 10. 96

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PLANT PHYSIOLOGY, vol. 105, no. 1, 1 May 1994, page 37 XP000561226 KROHN B M ET AL: "MODIFICATION OF STARCH STRUCTURE IN TRANSGENIC POTATO" see abstract 145 ---	27-41
A	WO,A,90 12084 (DNA PLANT TECHN CORP) 18 October 1990 see page 9, line 17 ---	44
A	ABSTRACTS VIITH INTERBATIONAL CONGRESS ON PLANT TISSUE AND CELL CULTURE, AMSTERDAM, JUNE 24-29, 1990, ABSTRACT NO. A5-28, page 177 XP002014046 VAN DER LEIJ, F.R., ET AL.: "Expression of the gene encoding granule bound starch synthase after introduction in an amylose-free and a wildtype potato (Solanum tuberosum)" see the whole document ---	1-26
A	WO,A,94 24292 (DANISCO ;VILLAND PER (NO); KLECZKOWSKI LESZEK (NO); OLSEN ODD ARNE) 27 October 1994 see page 29, line 25 - page 30, line 30 ---	40
E	WO,A,96 19581 (INST GENBIOLOGISCHE FORSCHUNG ;KOSSMANN JENS (DE); EMMERMANN MICHA) 27 June 1996 see the whole document -----	7,17, 23-26, 64,65

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